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AMINO ACID DIGESTIBILITY AND UTILIZATION OF PROTEIN SOURCES BY POULTRY

by

David John Summers

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

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ABSTRACT

The primary objective of this study was to try to develop procedures to obtain better estimates of amino acid digestibility of protein sources for poultry. For this purpose a series of experiments were conducted with four-week-old male broiler chicks to determine ileal and fecal amino acid digestibilities using procedures which involved obtaining samples of digesta from the ileum or collection of voided excreta. The ileal digesta were collected from the terminal 18 cm of the ileum of sacrificed birds. The excreta material was collected in trays beneath the birds before they were sacrificed. Experiments were also conducted with laying hens to evaluate the effects of dietary fat and calorie-protein ratio on protein utilization.

The amino acid digestibilities obtained using ileal digesta were similar to or lower than those obtained using fecal (excreta) material. Although not directly comparable, the total amino acid digestibilities of wheat, oats and barley were, 86.6%, 69.1% and 78.2%, respectively for ileal digesta and, 85.7%, 83.7% and 79.1%, for those determined by collection of fecal material. A direct comparison of ileal and fecal amino acid digestibilities of diets containing wheat, and either soybean meal or canola meal gave total digestibilities of, 89.2% and 94.9%, respectively, for soybean meal containing diets and 81.4% and 90.4%, respectively, for canola meal containing diets. Similar differences between total ileal and fecal amino acid digestibilities were observed when soybean meal and canola meal were used as the only protein source in the diets fed. In an experiment (Chapter VI) soybean meal had ileal and fecal amino acid digestibilities of 83.0% and 87.4%, respectively and canola meal had digestibilities of 76.0% and 85.8%. The results suggest that the protein sources of lesser digestibility, such as canola meal, tend to have greater differences between the ileal and fecal amino acid values.

The differences noted between the ileal and fecal amino acid digestibilities may be related to the activity of the hindgut microflora in poultry. Deamination of unabsorbed amino acids entering the hindgut by the microflora may lead to an increase in the apparent digestibilities of the amino acids when fecal collections are used to determine digestibility. For this reason values obtained using sampling of the ileal digesta should be more reliable.

In an attempt to obtain better estimates of endogenous amino acid excretion, diets containing graded levels of the protein sources were fed. It was observed, however, that when diets contained more than 15-20% protein, the regression between amino acid digested and amino acid intake no longer remained linear because a decrease in the digestibility of the amino acids occurred at higher levels of protein intake. With such a decrease in digestibility at higher protein intakes no single estimate of amino acid digestibility was possible. For this reason digestibility values obtained using a curvilinear equation may be more accurate.

Arising from these studies it became apparent that the major problem in accurately determining amino acid digestibilities is the lack of knowledge of endogenous amino acid excretions. As a result, the estimates of total amino acid digestibility may be slightly high or low. Despite the inaccuracies that may have occurred, information on the digestibilities of the individual amino acids as well as the total amino acid digestibilities obtained are of value in formulating poultry rations.

Two experiments were conducted with laying hens to evaluate the influences of dietary tallow levels on protein utilization and to assess the influence of calorie-protein ratio on egg weight and egg production. In the first experiment four levels of tallow (0, 3, 6, and 9%) were added to a series of diets with two calorie-protein ratios (190:1 and 210:1 kcal ME /%crude protein /kilogram of diet) and two sources of supplementary protein; soybean meal or canola meal. No significant effects of level of tallow on rate of egg production or egg weight were observed. There was, however, a significant increase in body weight-gain with higher levels of tallow in the diet. This was attributed to increased energy utilization rather than increased protein utilization. There was a significant difference in calorie and protein consumption between the two calorie-protein ratios. This had no effect on rate of egg production but a significant decrease in egg weight (56.8 versus 55.7) was observed with the wider (210:1) calorie-protein ratio. The hens fed diets containing canola meal had significantly lower egg weights and lower rate of egg production than those fed diets containing soybean meal. Energy and protein intakes were also lower on the canola meal diets.

In the second experiment with laying hens, isocaloric diets containing wheat and either soybean meal or canola meal were designed to provide four calorie-protein ratios (190:1, 200:1, 210:1 and 220:1). A trial was also conducted to determine ileal and fecal amino acid digestibilities of the feed ingredients. The hens consumed equivalent levels of calories and protein within each calorie-protein ratio on the diets containing either soybean meal or canola meal. As the calorie-protein ratios were widened there was a decrease in egg production with both protein sources; there was also a significant decrease in egg weight. Ileal and fecal corrected amino acid digestibility values determined on the feedstuffs were 74.7 and 79.2%, respectively, for canola meal, 91.6 and 92.9% for soybean meal and, 84.7 and 96.3% for wheat. The decrease in amino acid digestibility of canola meal explained most of the decrease in egg production (86.7 versus 84.3 % /hen /day) and egg weight (58.7 versus 57.6 grams) of the hens on the diets containing canola meal, demonstrating that digestible amino acid content provides a better estimation of the nutritional value of the amino acid content in diet formulation, versus the use of total amino acid content.

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I. THESIS INTRODUCTION

In recent years there have been marked improvements in the efficiency of poultry production. One of the many contributing factors involved in this improved efficiency is the greater knowledge of the nutrition of poultry. Efficient production of poultry requires the use of rations that meet their nutrient requirements without providing excess amounts that may be wasted. This is particularly true of the amino acid component of the diet In formulating poultry rations provision of adequate levels of amino acids in the diet is essential to efficient production of poultry meat and eggs. This makes the knowledge of the amino acid content and its digestibility in feedstuffs very important when formulating poultry rations that will give optimum productivity.

In the past decade many different methods have been used to try to achieve estimates of the amino acid digestibilities of poultry feedstuffs, resulting in digestibility values which tend to vary depending on the procedure used to obtain them. In particular a procedure involving force-feeding of a feedstuff followed by total excreta collection during the subsequent 24 to 48 hr has become popular. To correct for endogenous amino acids the total excreta are collected from birds that have been starved for the same 24 to 48 hr period. There has been some criticisms of this method because of the unknown effects of factors such as alteration of the amino acids in the hindgut by microflora and the possible contamination of fecal amino acids by urinary amino acids. It therefore seemed desirable that studies be conducted using ileal digesta collection for amino acid determination, thus eliminating the influence of the hindgut on amino acid digestibility. Consequently studies were undertaken to compare the levels of amino acids in ileal digesta and excreta and their influence on the determination of the amino acid digestibilities of feedstuffs. In addition other studies were conducted with laying hens to evaluate the effects of dietary fat and calorie-protein ratio on protein utilization. The experiments conducted with broiler chickens are outlined in Chapters 2-7 and with laying hens in Chapters 8 and 9.

II. The Amino Acid Digestibility of Cereals in Broiler Chicks as Determined using Total Fecal Collections

A. Introduction

The digestibility of amino acids (AA) in feedstuffs is closely related to the digestibility of the protein. Consequently numerous attempts have been made to determine the protein digestibility in feedstuffs (Payne et al.1968; Waring 1969; Netke and Scott 1970; Tao et al.1971; Shannon and McNab 1973; McNab and Shannon 1974; Sibbald 1979a and Robel 1980). One of the difficulties in determining the true digestibility of AA is that an accurate estimation of endogenous AA excretion is required. Since no direct method for measuring endogenous AA has been devised, estimates based on the AA excretion on N-free diets ,on diets containing graded levels of dietary AA, or by starved birds have been used (Berdanier et al.1967; Shannon and McNab 1973; Sibbald 1979a).

Cereals contribute approximately 50% of the protein source in poultry rations and therefore more precise information on the AA digestibility of cereals should lead to increased precision in formulating diets for poultry. The present study was undertaken to investigate AA digestibility values for wheat, oats, and barley grown in Western Canada.

B. Materials and Methods

Day old male broiler chicks (White Mountain o x Hubbard +) were reared on a commercial type ration to 4 weeks of age and then transferred to growing batteries with raised wire floors. The cages (76cm x 76cm) were equipped to allow for quantitative feeding and collection of excreta. Birds were housed three to a cage and given 16 hr of light per day.

The composition of the experimental diets used is presented in Table II.1. In the experimental diets wheat, oats, and barley were substituted for either 30, 60, or 90% autoclaved corn starch in a N-free diet. The N-free diet contained 90% autoclaved corn starch. The autoclaved corn starch was mixed with equal quantities of water, autoclaved for 90 minutes at

120°C, dried and ground. This was done to improve the texture of the starch to enhance palatability.

Prior to the experimental period the birds were fasted for 12 hr after which 3 replicate pens of chicks were placed on each of the 10 experimental diets. The diets were fed ad libitum in mash form for 84 hr after which the birds were again fasted for the final 12 hr of the test period. Excreta collection commenced after the initial 12 hr fast and continued for 96 hr, terminating at the end of the final 12 hr fast. The excreta were collected at the end of each 24 hr period and were placed in foil containers and frozen, then freeze dried, pooled per replicate and weighed. Feed consumption and body weight changes were recorded at the conclusion of the trial.

Diets and excreta were freeze-dried and ground in a Wiley mill with a .42 mm mesh screen before analysis of dry matter and AA content. Amino acid contents were determined in duplicate using the method outlined by Blackburn (1968). Samples were hydrolyzed by refluxing with 6N HCl for 24 hr and their AA content was determined using a Beckman 121MB AA analyzer. The AA content of the cereals used is contained in Appendix XII.1.

The grams of AA absorbed were regressed on the grams AA intake on the diets represented by the levels of 0, 30, 60, and 90% cereal substitution. The slopes of the linear regressions through these four points were used to derive digestibility values for the AA. The digestibility values for the individual AA are expressed as percentages and the total CAAD is obtained using the weighted average of the individual CAAD. Estimates of endogenous AA excretion were obtained by two methods. In the first method the intercept of the regression slopes at zero AA intake was used. In the other method the amount of AA excreted on the N-free diet was used.

C. Results and Discussion

The influence of treatments on mean feed consumption, excreta production and body weight change during the 96 hr of test is summarized in Table II.2. Average excreta excretion was related to feed consumption. There was, however, considerable variation in the relationship and this variation was responsible for some of the differences in the amount of total AA excreted.

Estimates of corrected amino acid digestibility (CAAD) are presented in Table II.3. Wheat had the highest total CAAD (85.7%), followed by oats (83.7%), and barley (79.1%). These CAAD values compare favorably to the values reported by McNab and Shannon (1974) of 84.7, 85.0, and 83.6% for wheat, oats, and barley respectively. There was however considerable variability in the digestibility of individual amino acids. Lysine and threonine digestibilities were lower than total CAAD in all three grains. The lysine digestibility values for wheat, oats, and barley were 70.5, 74.1, and 68.5% respectively, while the digestibility values for threonine were 71.4, 71.4, and 74.2%. Digestibility values for glutamic acid, proline, and phenylalanine were higher in comparison to total CAAD (Table II.3). The digestibility of the other AA tended to be close to the total CAAD values for that particular grain.

The digestibility values of lysine reported by McNab and Shannon (1974), were 80.8, 75.7, and 75.4% for wheat, oats, and barley and those reported by Sibbald (1979b) were 91, 86, and 79%. While these values differ considerably from the digestibility values that were observed in the present study, their lysine digestibility values were consistently lower than total CAAD in all of the cereals which is consistent with our present observations. The same pattern was noted for threonine. The threonine digestibility values reported by McNab and Shannon (1974) of 72.1, 79.9, and 76.1% for wheat, oats and barley, and Sibbald (1979b) of 91, 82, and 78% were consistently lower than total CAAD. Proline and glutamic acid showed similar trends except their CAAD were consistently higher than total CAAD of the particular cereal. The other AA tended to follow the same pattern with individual AA having similar variation from the total CAAD within a particular cereal. This suggests that although differences in methodology may

alter absolute values, the relative digestibilities of the individual AA in comparison to total CAAD tend to follow a similar pattern.

Even though the standard error of the CAAD values obtained in the present experiment was low (Table II.3), the values did not always agree with others that have been reported (McNab and Shannon, 1974; Sibbald 1979b). Various reasons for the difference may be suggested. The composition of the cereals used may vary markedly amongst varieties causing differences in AA digestibility estimates (Sauer et al., 1981). Differences in methodology may influence the results obtained. McNab and Shannon (1974) used mature colostomised cockerels and a linear regression was used to correct for endogenous AA, whereas Sibbald (1979b) used intact mature cockerels and corrected for endogenous AA by using a 24 hr fast period. Variations in the magnitude of the correction for endogenous AA can lead to variation in the magnitude of the CAAD. Other minor alterations in methodology may also have affected the derived values. Even analysis of AA between laboratories may show considerable variation as reported by Engster (1983). The net effect is that with the individual variations that may exist it is difficult to determine which values are more accurate or which methodology practices are correct.

One of the problems in determining CAAD is correcting for endogenous AA excretion. The intercept values of the linear regressions at zero AA intake may be used to obtain an estimate of endogenous AA excretion or a measurement of the AA excreted on the N-free diet may also be used to estimate endogenous AA excretion directly. In this experiment estimates of endogenous AA excretion obtained tended to vary only a small amount with either method used (Table II.4). When the intercept of the linear regression was used the barley diets gave AA excretion values which were similar or slightly lower than those obtained when values were derived with the N-free diet. For diets containing wheat or oats the endogenous AA values derived with a linear regression were similar or slightly higher than those obtained with the N-free diet. Nevertheless differences in estimates of endogenous AA excretion with the two methods indicated that differences may exist between CAAD values depending on which

method was used. In this regard it has been suggested that values obtained with a N-free diet may be in error because such values assume the same level of endogenous excretion for all diets (Krawielitzki and Bock 1976). Waring (1969) and Muztar and Slinger (1981), demonstrated that body weight and dry matter intake could influence endogenous amino acid excretion. In the present study the influence of intake on AA excretion was apparent. Intakes of 217, 318 and 257 grams N-free diet gave total amino acid excretions of 5.36, 7.35 and 5.23 grams during the 96 hr collection period. Such data confirm that level of feed intake may influence the estimate of endogenous AA when using N-free diets. Thus, ensuring constant dry matter intake for all treatments should help to avoid some of the errors caused by differences in AA excretion levels. This however creates the problem of selecting the level of intake that gives the most accurate estimation of endogenous AA excretion. Inaccuracies can occur with all methods presently used to correct for endogenous AA excretion and therefore it is difficult to directly compare CAAD values derived using different methods.

D. Summary

The results obtained indicated the wheat had the highest total CAAD (85.7%), followed by oats (83.7%), and barley (79.1%). The digestibility of lysine and threonine was lower than total CAAD in all three cereals, but the digestibilities of glutamic acid, proline and phenylalanine were all higher than total CAAD. The estimates of endogenous AA excretion varied depending on whether they were obtained by the regression method or by measuring excretion on the N-free diet

Table II.1 Composition of the experimental diets.

Ingredients %	Diet No. 1	2	3	4	5	6	7	8	9	10
Corn starch	90	60	30	-	60	30	-	60	30	-
Ground wheat	-	30	60	90	-	-	-	-	-	-
Ground oats	-	-	-	-	30	60	90	-	-	-
Ground barley	-	-	-	-	-	-	-	30	60	90
Constant ingredients[1]	10	10	10	10	10	10	10	10	10	10
Analyses:										
Moisture %	9.0	9.6	11.6	11.6	9.4	10.3	10.8	10.4	11.7	13.7
Crude protein %	0.76	4.76	9.11	13.56	3.55	6.19	8.03	3.63	6.75	9.53

[1]Supplied the following levels per kg of diets: corn oil 42g; ground limestone 8g; Biofos (18% Ca-21% P) 33g; iodized salt 6g; vitamin-mineral premix 11g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D_3 600 ICU; vitamin E 10 IU; menadione 3mg; riboflavin 6mg; calcium pantothenate 10mg; niacin 15mg; folic acid 1 mg; biotin .2mg; vitamin B_{12} 10mcg; choline chloride 1500mg; manganese 100mg; zinc 80mg.

Table II.2 Influence of treatment on feed consumption, excreta production and change in body weight of broilers.

Diet	Treatment	Avg. feed[1] consumed g	Avg. excreta[2] production g	Avg. body weight change g
1	N-free	88	20	-108
2	Wheat 30%	174	35	-68
3	Wheat 60%	226	54	6
4	Wheat 90%	195	58	24
5	Oats 30%	147	35	-66
6	Oats 60%	244	73	-23
7	Oats 90%	279	105	35
8	Barley 30%	180	41	-52
9	Barley 60%	279	82	1
10	Barley 90%	317	112	44

[1]Feed consumed/ chick/ 84 hr feeding period.

[2]Excreta excreted/ chick/ 96 hr collection period.

Table II.3 Fecal amino acid digestibility of cereal grains obtained using the linear regressions.

Amino acid	Digestibility		
	Wheat %	Oats %	Barley %
Aspartic acid	77.2 ± 1.4[1]	82.4 ± 1.5	69.9 ± 1.7
Threonine	71.4 ± 2.5	71.4 ± 2.0	74.2 ± 1.3
Serine	84.8 ± 1.2	80.4 ± 1.8	72.9 ± 1.7
Glutamic acid	94.6 ± 1.4	94.0 ± 1.3	87.1 ± .5
Proline	92.6 ± 1.4	82.7 ± 1.4	84.9 ± .9
Alanine	76.4 ± 2.3	75.4 ± 1.0	70.1 ± 2.3
Valine	78.0 ± 4.4	77.4 ± 2.7	79.7 ± 1.7
Methionine	84.9 ± 6.6	48.4 ± 3.7	75.6 ± 2.0
Isoleucine	74.4 ± 1.9	83.1 ± 1.5	78.7 ± 1.1
Leucine	75.8 ± 1.9	79.3 ± 1.7	77.0 ± 1.8
Tyrosine	81.2 ± 4.2	85.1 ± 3.5	74.8 ± 1.5
Phenylalanine	89.3 ± 2.5	87.0 ± 1.1	80.4 ± 1.2
Lysine	70.5 ± 2.7	74.1 ± 1.8	68.5 ± 2.1
Histidine	86.6 ± 2.2	87.0 ± 1.3	79.4 ± 1.0
Arginine	86.4 ± 1.0	91.3 ± 1.4	77.5 ± 1.2
Total	85.7 ± 1.1	83.7 ± 1.2	79.1 ± 1.0

[1]Digestibility coefficient ± standard error.

Table II.4 Estimates of endogenous amino acid excretion (mg/bird/day) using the N-free diet and regression intercepts.

Amino acid	Intercept Values of Regression			
	N-free	Wheat	Oats	Barley
Aspartic acid	52.9 ± 4.6[1]	56.0 ± 4.3	59.6 ± 6.7	51.5 ± 7.2
Threonine	32.3 ± 2.6	34.6 ± 4.4	32.3 ± 3.7	31.1 ± 3.1
Serine	41.7 ± 4.3	42.4 ± 3.9	47.1 ± 4.8	40.2 ± 5.0
Glutamic acid	71.1 ± 7.1	79.1 ± 8.4	88.7 ± 14.7	64.7 ± 8.1
Proline	39.8 ± 4.1	45.5 ± 9.5	43.1 ± 4.8	31.8 ± 5.6
Alanine	31.4 ± 3.2	31.6 ± 5.1	30.8 ± 3.4	31.0 ± 6.3
Valine	35.2 ± 2.6	33.6 ± 11.6	29.1 ± 7.9	29.4 ± 5.6
Methionine	9.7 ± .7	11.6 ± 5.2	4.4 ± 3.0	7.7 ± 2.3
Isoleucine	23.1 ± 2.5	25.8 ± 4.2	29.1 ± 3.0	21.5 ± 2.6
Leucine	46.6 ± 5.4	59.5 ± 8.1	53.9 ± 6.6	37.1 ± 4.1
Tyrosine	26.7 ± 2.7	31.9 ± 5.5	32.1 ± 4.5	26.3 ± 2.4
Phenylalanine	26.9 ± 2.8	29.2 ± 7.3	29.5 ± 3.0	25.9 ± 4.2
Lysine	41.4 ± 8.6	43.8 ± 4.3	42.4 ± 4.3	38.4 ± 5.0
Histidine	13.8 ± 1.5	14.8 ± 3.0	14.8 ± 1.5	12.5 ± 1.5
Arginine	27.8 ± 2.6	28.1 ± 2.7	35.8 ± 4.5	26.9 ± 3.7
Total	520.7 ± 86.6	605.1 ± 46.9	590.4 ± 69.6	476.8 ± 30.2

[1]Intercept ± standard error.

III. Comparison of Apparent Amino Acid Digestibilities in Anesthetized and Sacrificed Chickens Using Diets Containing Soybean Meal and Canola Meal.[1]

A. Introduction

To achieve an estimate of amino acid digestibility of feeds using chickens the levels of amino acids present in the intestinal contents of sacrificed birds have been compared to the levels provided in the feed as a basis for the calculation (Imondi and Bird, 1965; Payne et al., 1968; Crompton and Nesheim, 1969; Soares and Kifer, 1970; Achinewhu and Hewitt, 1979; Raharjo and Farrell, 1981). In previous studies with sheep, Badawy (1964) suggested that such an estimate might be inaccurate because when sacrificed animals were used the intestinal contents might be contaminated with cells shedding from the lining of the intestine. The presence of the additional amino acids represented by the cell shedding could thus cause the apparent amino acid digestibility (AAAD) of the feed to be underestimated. Other studies with pigs (Thorpe and Thomlinson, 1967) and calves (Pearson and Logan, 1978) indicated that the degree of cell shedding increased with time after death, as well as with the site within the small intestine in which the samples were taken.

Because the reliability of estimates of AAAD might be influenced by alteration of endogenous amino acid levels due to cell shedding, an experiment was undertaken to determine whether the accuracy of estimation of apparent amino acid digestibility could be improved by using anesthetized instead of sacrificed chickens. In addition, samples of intestinal contents obtained from different segments of the terminal ileum and from the large intestine of birds fed different rations were used to determine whether site of sampling or feed type would affect estimation of AAAD with either anesthetized or sacrificed chickens.

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[1]A similar version of this chapter has been accepted for publication in Poultry Science 1985.

B. Materials and Methods

Male broiler chicks (White Mountain o x Hubbard +) were raised in a Petersime chick battery and fed a commercial-type diet to three weeks of age. Birds of comparable weight were then randomly assigned to 6 groups of 7 birds each and transferred to growing cages (76cm x 76cm) with raised screen floors. Feed and water were provided ad libitum and the birds received 16 hours of light per day.

The composition of the experimental diets is given in Tables III.1 and III.2. The protein supplements consisted of either soybean meal, soybean meal and canola meal, or canola meal. Cellulose was included in diets 1 and 2 to keep the level of fiber comparable to that in diet 3 and all diets were formulated to be isonitrogenous. Chromic oxide was added as an inert marker. Two groups of chicks were placed on each of the experimental diets, one group to be used as anesthetized birds and the other group as sacrificed birds.

For digesta collection the birds to be anesthetized received 5% halothane for immobilization and 2% halothane for maintenance of immobilization. The sacrificed birds were killed by cervical dislocation. In both cases as soon as the birds had been immobilized the abdomen was opened and the ileum removed. Digesta were collected from segments 0-6, 6-12, and 12-18 cm anterior to the ileocecal junction and from the large intestine. The contents of each segment were gently squeezed into a plastic container. Following the removal of the ileum the anesthetized birds were killed by cervical dislocation. The time required either to anesthetize or sacrifice and to collect the digesta was approximately seven minutes. Collections were made from 10:30 to 11:30 on days 3, 4, 5 and 6 after being placed on the experimental diets. Two birds from each pen were used on days 3, 4 and 5 and one bird on day 6. The samples collected from each pen throughout the experiment were pooled to provide sufficient material for analysis.

The digesta were freeze-dried ,weighed and and ground in a Universal micro mill for 5 minutes. Dry matter, chromic oxide and amino acid content were determined on both feed and digesta samples. Chromic oxide was determined by the method of Fenton and Fenton (1979).

The amino acid analyses of the feed and intestinal samples were done in duplicate using the method outlined by Blackburn (1968). The samples were hydrolyzed by refluxing with 6N HCl for 24 hr and their amino acid content was determined using a Beckman 121 MB amino acid analyzer.

Apparent amino acid digestibilities were calculated using the following equation:

$$AAAD = \frac{\text{feed a.a.} - (\text{feed } Cr_2O_3/\text{fecal } Cr_2O_3 \times \text{fecal a.a.}) \times 100}{\text{feed a.a.}}$$

Data were subjected to analysis of variance. Significance of differences among sources of variation; diets, method of collection and site of collection were determined by using the three factor interaction as error variation. Differences among means were computed using Student-Newman-Keuls' procedure (Steel and Torrie, 1980) at the 0.05 level of probability.

C. Results and Discussion

The effects of digesta collection on AAAD are presented in Table III.3. No significant differences in AAAD were noted between anesthetized and sacrificed birds except for five amino acids (alanine, aspartic acid, glutamic acid, lysine and serine) that showed differences at the 5% level of probability. However, the anesthetized chickens consistently showed slightly higher AAAD values than the sacrificed chickens. The overall average increase in digestibility with anesthetized chickens was 1% unit (83.9 vs 82.9). If the digesta were contaminated with extra amino acids from endogenous sources it would have decreased apparent digestibility. Thus, the lower digestibility observed with the sacrificed chickens may possibly have been influenced by cell shedding. In this regard Thorpe and Thomlinson (1967) using pigs found epithelial cell shedding increased with time after death. The site of sampling in the small intestine was also shown to influence the time at which cell shedding started. Cell shedding at the ileum was shown to take longer to start than at the duodenum. Similar results for epithelial cell shedding were noted by Pearson and Logan (1978) using exsanquinated calves in which samples were taken from 1 to 30 minutes after severing the carotid arteries. Epithelial cell

separation was nil or minimal up to 10 minutes. Pearson and Logan (1978) also took samples from the proximal, middle and distal sections of the small intestine and found epithelial cell shedding took longer to commence at the distal section of the small intestine than at the proximal section (15 vs 5 minutes). In the present experiment, samples were collected within seven minutes from the ileum. If the chicken reacts in the same way as pigs and calves, cell shedding should not have started or should have been minimal in the ileum at seven minutes after sacrifice.

In the present experiment, upon death, most of the sacrificed birds showed peristaltic contractions of the intestinal tract which might have resulted in movement of digesta down the digestive tract. If such contractions moved partially digested material from the upper part of the intestinal tract into the distal ileum where the samples were taken this would have resulted in decreased apparent amino acid digestibility values. The anesthetized birds did not show any peristaltic contractions of the intestinal tract and therefore contamination of the terminal region by less digested contents should not have occurred.

Segments of the terminal 18 cm of the ileum were taken to see if any differences between AAAD values could be found in the different segments. In previous work using sacrificed birds (Payne et al., 1968; Soares and Kifer, 1970) half or whole segments of the ileum were used. As shown by Raharjo and Farrell (1981) the terminal 10 cm of the ileum had digestibility values greater than those obtained when using the whole ileum. The 18 cm of ileum used in this experiment represents approximately the terminal third of the total ileum.

No significant differences were noted for digestibility with any of the individual amino acids tested between the different segments of the terminal 18 cm of the ileum (Table III.3). Values derived using the contents of the large intestine although not significantly different from the ileal values, had slightly higher digestibility values. Digestibility values for the total amino acids were 83.1 ± 0.6, 83.4 ± 0.6, 83.1 ± 0.6 and 84.1 ± 0.6 for the sections 12 - 18, 6 - 12, 0 - 6 cm anterior to the ileocecal junction and the large intestine, respectively.

The lack of significant differences in AAAD between sacrificed and anesthetized chickens would not justify the extra time and expense of anesthetizing the chickens. In collecting digesta from the terminal ileum, it would be desirable to collect as much digesta as possible to reduce sampling variation and to ensure sufficient sample for analytical work. Because no significant variation was seen in samples taken from the three sections of the terminal 18 cm of the ileum, this suggests the terminal 18 cm could have been used to collect larger ileal samples without affecting the reliability of the digestibility data obtained.

Significant differences in AAAD due to diet were noted in the present study (Table III.4). Diet 3 which contained the highest level of canola meal, gave significantly lower digestibility values for many of the amino acids. This is in agreement with Muztar et al. (1980), who reported lower AAAD values for canola meal than soybean meal. The lack of a decrease in the digestibility of the AA in diet 2, containing 13% canola meal, cannot be explained.

D. Summary

Except for alanine, aspartic acid, glutamic acid, lysine, and serine, no significant differences in AAAD were found between anesthetized and sacrificed chicks, although the anesthetized chicks consistently showed higher values. No significant differences in AAAD resulted from taking digesta samples from different segments of the terminal ileum. The diets containing canola meal as the only protein supplement had lower AAAD than either diets with soybean meal or soybean meal and canola meal as the protein supplement. It was concluded that because of the small differences in AAAD between anesthetized and sacrificed chickens the time and expense associated with anesthetizing birds would not be justified.

Table III.1 Composition of experimental diets.

Ingredients %	Diet No. 1	2	3
Ground wheat	71.5	68.5	65.5
Stabilized tallow	3.0	3.0	3.0
Alfafloc[1]	2.0	1.0	-
Soybean meal	18.0	9.0	-
Canola meal	-	13.0	26.0
Constant ingredients[2]	5.5	5.5	5.5

[1]Alphafloc; Brown Company, Berlin, New Hampshire.

[2]Supplied the following levels per kg of diets: ground limestone 15g; Biofos (18% Ca-21% P) 12g; iodized salt 3g; chromic oxide 5g; vitamin-mineral premix 20g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D_3 600 ICU; vitamin E 10 IU; menadione 1mg; riboflavin 5mg; calcium pantothenate 10mg; niacin 20mg; folic acid 1mg; vitamin B_{12} 10mcg; choline chloride 1000mg; manganese 100mg; zinc 75mg.

Table III.2 Amino acid composition of diets.

Amino acid	Diet No. 1	2	3
	%	%	%
Aspartic acid	1.67	1.57	1.35
Threonine	.70	.72	.77
Serine	1.05	1.03	.96
Glutamic acid	5.45	5.25	5.23
Proline	1.72	1.74	1.78
Glycine	.91	.94	.97
Alanine	.78	.80	.78
Valine	.91	.94	.92
Methionine	.23	.25	.27
Isoleucine	.79	.78	.77
Leucine	1.49	1.45	1.44
Tyrosine	.44	.41	.47
Phenylalanine	.98	.92	.96
Lysine	.91	.90	.86
Histidine	.52	.45	.51
Arginine	1.18	1.14	1.08

TABLE III.3. Effect of digesta sampling site on apparent amino acid digestibilities using anesthetized or sacrificed chickens.

Digesta sampling site[1]	Anesthetized						Sacrificed						Collection Method SE
	Ileum 12-18 cm	Ileum 6-12 cm	Ileum 0-6 cm	Large intestine	Site SE	Mean[2]	Ileum 12-18 cm	Ileum 6-12 cm	Ileum 0-6 cm	Large intestine	Site SE	Mean[2]	
Amino acids	%												
Aspartic acid	78.2	77.7	78.7	81.1	±1.06	78.9	77.2	78.1	76.5	77.7	±1.06	77.4	±.52*
Threonine	74.8	73.7	74.4	77.8	±1.40	75.2	72.7	74.7	73.5	74.4	±1.40	73.8	±.70
Serine	81.3	80.3	81.2	83.6	± .54	81.6	79.5	80.3	79.6	81.2	± .54	80.1	±.38*
Glutamic acid	92.4	92.1	92.5	93.4	± .29	92.6	91.9	92.3	92.5	92.9	± .29	92.2	±.14*
Proline	88.7	88.2	88.8	89.9	± .59	88.9	87.6	88.5	88.0	88.5	± .59	88.2	±.30
Glycine	80.5	82.9	83.9	82.3	±1.19	82.4	82.6	83.3	78.8	79.2	±1.19	81.0	±.59
Alanine	82.2	81.9	81.9	84.2	± .77	82.6	80.1	81.7	80.2	80.7	± .77	80.7	±.39*
Valine	83.6	82.2	83.8	84.7	±1.33	83.4	80.3	83.0	82.1	82.9	±1.33	82.1	±.67
Methionine	90.2	90.3	88.4	88.6	±1.68	89.4	89.0	90.5	89.7	87.4	±1.68	89.1	±.84
Isoleucine	84.7	84.0	84.1	86.4	± .87	85.0	83.6	84.6	83.8	84.5	± .87	84.1	±.43
Leucine	84.6	84.9	84.8	86.8	±1.29	85.3	84.2	83.8	83.4	85.3	±1.29	84.2	±.42
Tyrosine	72.8	72.4	74.0	77.9	±1.33	74.3	71.7	73.4	72.2	73.9	±1.33	72.8	±.67
Phenylalanine	84.1	84.2	84.9	85.6	± .82	84.7	84.6	85.6	84.5	83.9	± .82	84.7	±.41
Lysine	86.7	85.1	85.2	86.8	±1.00	85.9	84.4	84.9	84.6	83.9	±1.00	84.4	±.50*
Histidine	86.8	85.9	86.2	87.9	± .66	86.7	85.5	86.3	85.3	86.2	± .66	85.8	±.33
Arginine	86.7	85.9	86.3	88.2	± .84	86.8	85.7	86.2	85.4	86.4	± .84	85.9	±.42
Total	83.6	83.2	83.6	85.3	± .80	83.9	82.5	83.7	82.5	83.0	± .80	82.9	±.40

[1]Digesta were collected from sections of the small intestine 12 to 18, 6 to 12, and 0 to 6 cm anterior to the ileocecal junction and the large intestine.

[2]Mean values for sacrificed chickens with an asterisk are significantly different from average values for anesthetized chickens at ($P<.05$).

Table III.4 Effect of diet on apparent amino acid digestibilities.

Amino acid	Diet No. 1	Diet No. 2	Diet No. 3	
	%	%	%	S.E.
Aspartic acid	79.5*a*	79.2*a*	72.9*b*	.64
Threonine	74.6*a*	75.7*a*	71.6*b*	.86
Serine	82.2*a*	80.5*b*	79.9*b*	.47
Glutamic acid	92.7	92.4	92.0	.18
Proline	90.0*a*	89.0*b*	86.5*c*	.36
Glycine	81.4*a*	81.0*a*	77.7*b*	.73
Alanine	80.4	82.1	81.0	.58
Valine	83.2*a*	83.2*a*	81.2*b*	.82
Methionine	90.2*a*	91.0*a*	87.6*b*	.86
Isoleucine	85.2	84.5	83.7	.53
Leucine	85.1*a*	84.6*a*	81.7*b*	.52
Tyrosine	75.6*a*	75.8*a*	71.6*b*	.81
Phenylalanine	84.9	85.2	84.0	.50
Lysine	86.0*a*	85.0*a*	85.8*b*	.75
Histidine	86.5*a*	86.5*a*	85.8*b*	.40
Arginine	86.9*a*	86.2*a*	84.7*b*	.63
Total	83.9*a*	83.8*a*	81.5*b*	.51

a,b,c Means followed by different subscripts are significantly different ($P < .05$).

IV. Amino Acid Digestibility of Cereal Grains in Broiler Chicks as Determined using Ileal Digesta Collections.

A. Introduction

In the preceding experiment (Chapter 3) estimation of apparent amino acid digestibility was made using digesta samples from the different segments of the terminal third of the ileum and from the large intestine. Since the site of sampling within the terminal third of the ileum had no effect on apparent amino acid digestibility it was concluded that in the procedure used the entire third of the terminal ileum could be used for digesta collection. The collection of digesta from the large intestine was excluded because of studies with swine and rats suggesting fecal amino acid digestibilities may be inaccurate due to microflora alteration of the amino acid (AA) profile in the hindgut (Mason and Palmer, 1973; Zebrowska, 1973; Eggum et al., 1979; Sauer et al., 1977). Since earlier estimates of corrected amino acid digestibility (CAAD) in wheat, oats and barley (Chapter 2) were made using total excreta collections it seemed desirable that the digestibility of these cereals be reassessed using the ileal collection procedure.

B. Materials and Methods

Male broiler chicks (White Mountain o x Hubbard +) were raised in a Petersime chick battery and fed a commercial-type diet. At four weeks of age, birds of comparable weight were randomly assigned to 30 groups of 3 birds each and transferred to growing cages (76cm x 76cm) with raised screen floors. The birds were provided with 16 hours of light per day.

The experimental diets (Table IV.1) contained levels of 0%, 30%, 60%, or 90% of wheat, oats or barley, with sucrose constituting the remainder of the diet up to a level of 90%. Ten percent of all diets consisted of fixed ingredients which included vitamins, minerals, corn oil and chromic oxide to serve as an inert marker.

Three pens of chicks were assigned to each of the experimental diets and the experiment was conducted over a three day period. One chick from each pen was sacrificed on each day and

the three collections from a pen were then pooled to achieve one sample per pen. For digesta collection the chicks were starved for 12 hr during the day before being placed on the experimental diets for 12 to 13 hr overnight. At the end of the 12 hr feeding period the chicks were sacrificed by cervical dislocation during the following hour, a diet at a time at random. The abdomen was then opened and the terminal 18 cm of the ileum used to collect ileal digesta, as described in Chapter 3.

The ileal digesta were freeze-dried, weighed and ground in a Universal micro mill for approximately 5 minutes before analysis. Dry matter, chromic oxide and AA content were determined in duplicate on both feed and digesta samples. Chromic oxide was determined by the method of Fenton and Fenton (1979). The AA analyses of the feed and intestinal samples were done using the method outlined by Blackburn (1968). The samples were hydrolyzed by refluxing with 6N HCl for 24 hr and their AA content was determined using a Beckman 121 MB amino acid analyzer. The AA content of the cereals used in this chapter and protein sources used in subsequent chapters are contained in Appendix XII.1.

The concentration of chromic oxide in the digesta was used to determine the concentration of AA in the digesta in relation to feed intake. Based on a 100 grams dietary intake, regressions were determined between the amount of AA intake and the amount of AA absorbed. To determine if quadratic regressions might give a significantly better fit then linear regressions step-wise quadratic regressions were also computed. The digestibility coefficients were then determined by using the derivative of the regressions to obtain AA digestibility values that were corrected for endogenous AA excretion.

C. Results and Discussion

The quadratic regressions did not have a significantly better fit then the linear regressions and therefore only CAAD derived from the linear regressions are presented (Table IV.2). The intercepts of the linear regressions which were used to calculate CAAD are contained in Appendix XII.2. There were substantial differences in CAAD among the different

cereals with total CAAD of 86.6%, 69.1%, and 78.2% for wheat, oats, and barley respectively. There was however considerable variability in the digestibility of individual AA within the different grains. The range of digestibility of individual AA in wheat was 76.1% to 93.3%; in oats 51.8% to 82.3%; and in barley 71.1% to 85.3%. Unfortunately a direct comparison to the data in Chapter 2 cannot be made because of differences in the sources of cereals used. In addition sucrose was used instead of autoclaved corn starch as the N-free carbohydrate source in the diet. Sucrose was substituted for autoclaved starch in this experiment in an effort to standardize the determination by having a non-variable N-free carbohydrate source; however, there was more feed spillage on the diets containing sucrose and the feces were very wet which would make collection of fecal material more difficult.

There were some elements of agreement between the results in Chapter 2 and the present experiment which are worth noting even though there were differences in the cereal sources and the N-free carbohydrate source. The total CAAD of the wheat and barley were similar in both experiments. Wheat and barley had ileal digestibilities of 86.6% and 78.2%, respectively as compared to fecal CAAD observed in Chapter 2 of 85.7% and 79.1%, respectively. The total CAAD for oats was markedly different between the experiments, 69.1% for ileal and 83.7% for fecal digestibility. Because different sources of oats were used no explanations for the differences can be suggested. In wheat and barley the digestibility of individual AA was similar in both experiments. Aspartic acid, threonine and lysine had individual CAAD below the total CAAD, and glutamic acid and proline had digestibilities above total CAAD. The pattern of digestibility of individual AA in oats showed much more variation than in the case of wheat and barley. Achinewhu and Hewitt (1979) using ileal digesta found similar patterns in the digestibility of individual AA in wheat and barley. Methionine, glutamic acid, and proline were digested to the greatest extent while lysine, aspartic acid, threonine, serine, alanine, and histidine were slightly less well digested.

Two of the essential AA, lysine and threonine, were lower in digestibility than total CAAD. This is of particular concern when formulating practical poultry rations because these

are two AA that are likely to become limiting in the diet It is therefore important that attention be given to their digestibility as well as the digestibility of the total AA in diet formulations. The digestibility of other essential AA was close to that of total CAAD, consequently, a measurement of total protein digestibility gives a reasonable estimate of the digestibility of these AA.

An overall evaluation of the data derived using ileal digesta indicated that the ileal CAAD values were similar to the fecal values derived in Chapter 2. Thus using the ileal sampling procedure appeared to give satisfactory estimates of CAAD.

D. Summary

The results obtained indicated wheat had the highest ileal CAAD (86.6%), followed by barley (78.2%), and oats (69.1%). The digestibility of aspartic acid, threonine and lysine in wheat and barley was below total CAAD, while glutamic acid and proline had digestibilities above total CAAD. Oats had greater variation in the digestibilities of the individual AA with lysine, threonine, serine, alanine and histidine having lower digestibilities, while methionine, glutamic acid, and proline had digestibilities above total CAAD.

TABLE IV.1. Composition of experimental diets.

Ingredients %	Diet No. 1	2	3	4	5	6	7	8	9	10
Sucrose	90	60	30	-	60	30	-	60	30	-
Ground wheat	-	30	60	90	-	-	-	-	-	-
Ground oats	-	-	-	-	30	60	90	0	0	-
Ground barley	-	-	-	-	-	-	-	30	60	90
Constant ingredients[1]	10	10	10	10	10	10	10	10	10	10

[1]Supplied the following levels per kg of diets: corn oil 32g; ground limestone 8g; Biofos (18% Ca-21% P) 33g; iodized salt 6g; chromic oxide 10g; vitamin-mineral premix 11g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D_3 600 ICU; vitamin E 10 IU; menadione 3mg; riboflavin 6mg; calcium pantothenate 10mg; niacin 15mg; folic acid 1 mg; biotin .2mg; vitamin B_{12} 10mcg; choline chloride 1500mg; manganese 100mg; zinc 80mg.

Table IV.2 Ileal amino acid digestibility of cereal grains obtained by using linear regressions.

Amino acid	Digestibility		
	Wheat %	Oats %	Barley %
Aspartic acid	76.1 ± 2.4[1]	66.4 ± 2.9	71.1 ± 2.0
Threonine	79.8 ± 1.6	51.8 ± 3.9	73.0 ± 2.4
Serine	85.4 ± 1.7	56.7 ± 4.1	73.2 ± 2.4
Glutamic acid	93.3 ± .8	82.3 ± 1.6	85.3 ± .7
Proline	92.1 ± 1.1	62.9 ± 3.2	82.9 ± 1.1
Glycine	77.4 ± 2.6	58.2 ± 4.9	68.2 ± 2.0
Alanine	78.3 ± 2.1	62.1 ± 3.9	71.2 ± 3.6
Valine	81.1 ± 2.0	68.1 ± 3.3	75.8 ± 1.7
Methionine	86.5 ± 1.4	77.6 ± 8.8	75.9 ± 2.0
Isoleucine	85.7 ± 1.3	71.8 ± 4.5	77.7 ± 1.4
Leucine	87.1 ± 1.1	74.3 ± 1.6	80.4 ± 1.1
Tyrosine	83.1 ± 1.9	59.7 ± 4.7	73.8 ± 2.2
Phenylalanine	87.3 ± 1.1	69.5 ± 4.0	78.9 ± 1.7
Lysine	76.7 ± 2.3	64.6 ± 3.1	73.1 ± 1.6
Histidine	81.3 ± 1.6	63.3 ± 4.0	74.5 ± 1.8
Arginine	80.2 ± 2.0	68.2 ± 3.6	74.3 ± 2.2
Total	86.6 ± 1.2	69.1 ± 2.5	78.2 ± 1.3

[1]Digestibility coefficient ± standard error.

V. Estimates of Ileal and Fecal Amino Acid Digestibility in Broiler Chicks

A. Introduction

In the past decade research using pigs has established that amino acid digestibilities derived using fecal material may be in error due to the influence of the hindgut microflora population. The microflora can alter the amino acid (AA) profile of the ileal digesta through AA synthesis or deamination (Mason and Palmer, 1973; Eggum et al., 1979; Low, 1979). Since amino acids or peptides in the hindgut cannot be absorbed into the blood stream they can only be deaminated to serve as a source of nitrogen to the pig (Deguchi et al., 1978; Rerat, 1978; Wunsche et al., 1982). As a consequence the use of ileal AA digestibility have been suggested as being a more accurate estimation of true AA digestibility rather than the digestibilities derived by collection of fecal (excreta) material (Payne et al., 1968).

In poultry the question of whether ileal AA digestibility values are more accurate then fecal AA digestibility values has been a contested issue. Poultry are similar to pigs in that they have no absorption of AA from the hindgut (Salter and Coates, 1971; Furuse and Yorkota, 1984). The argument with poultry has been, that with the rapid passage of the digesta, the microflora do not have enough time to alter the AA profile and therefore fecal AA digestibility should be as accurate as ileal AA digestibility . An experiment conducted by Parsons et al. (1982) reported that in poultry 25% of fecal AA were of microbial origin as compared to 50% in swine. Although the proportion is much lower, the microflora still had a significant influence upon the pattern of AA excreted in the excreta with the greatest influence being in estimates of endogenous AA excretion.

Other studies have also evaluated the effect of the hindgut of poultry on AA digestibility. Studies using cecectomized birds have indicated an increase in the level of AA excreted on N-free diets or while fasting as compared to intact birds (Kessler and Thomas, 1981; Parsons, 1984). Nesheim and Carpenter (1967) using cecectomized versus normal chicks observed a 10% decrease in the AA digestibility of heat-damaged cod muscle. Using germ-free

chicks Salter and Fulford (1974) found that the gut microflora had little influence on the digestibility of proteins. In a few experiments comparing ileal and fecal AA digestibility directly, the ileal digestibilities have been lower, especially with less digestible protein sources (Varnish and Carpenter, 1975; Raharjo and Farrell, 1981).

Since there was some question of the influence of the hindgut on AA digestibility, a study was conducted to compare the levels of AA in the ileal and fecal digesta and their effects on AA digestibility. Soybean meal and canola meal were used in the experiment to see if the differences in AA digestibility observed previously (Chapter 3) would have an influence on the level of AA found in the ileal and fecal digesta. In addition the influence of the length of fasting and the length of the feeding period was examined to determine their possible effects on the level of AA collected.

B. Materials and Methods

The breed of chickens used, their management and the procedure followed in the collection of the ileal digesta in this experiment were similar to those described in Chapter 4. The only difference was the birds were provided with continuous lighting. The fecal samples were collected in trays beneath the cages during the final 2 hr of the feeding period before the chicks were sacrificed. To test the effects of length of the fasting time the birds were starved for 12, 24, or 36 hr before being fed for a 12 hr feeding period. To assess the effect of length of the feeding period the birds were starved for 12 hr before being fed for a 12, 24, 48 or 72 hr period.

The experimental diets (Table V.1) consisted of wheat and either soybean meal or canola meal as a protein source in the diet Each protein source was added to the diets at graded levels (0, 30, 61, or 91%) replacing autoclaved corn starch. At each level in the diet the proportion of wheat to soybean meal or wheat to canola meal was kept constant. Nine percent of all diets consisted of fixed ingredients which included vitamins, minerals, corn oil and chromic oxide.

The laboratory and statistical analysis conducted were the same as those described in Chapter 4.

C. Results and Discussion

The corrected amino acid digestibility (CAAD) determined using the derivatives of the linear regressions between the grams AA digested versus the grams feed intake are presented in Table V.2. The intercepts of the linear regressions are contained in Appendix XII.3. The CAAD values noted when ileal collections were used were lower than the values derived from the fecal collections. The digestibility of the wheat-soybean meal diet was higher than that of wheat-canola meal diet. The differences in the digestibility values between the wheat-soybean meal and the wheat-canola meal diets were greater when measured using ileal as compared to fecal collections.

The plotted values used to derive the linear regressions appeared to have a curvilinear relationship and consequently quadratic regressions of the data were derived. The quadratic regressions for the total AA and their derivatives are presented in Table V.3. The regressions for the individual AA are contained in Appendices XII.4, 5, 6, and 7. A number of the individual and total AA quadratic regressions were found to be have a significantly better fit then the corresponding linear regressions. The significance of the total AA quadratic regressions as compared to the linear regressions is indicated in Table V.3. When the fecal digesta were used for the calculations wheat-soybean had a highly significant quadratic regression ($P = .0002$); however, when the ileal digesta were used the quadratic regression was highly non-significant ($P = .929$). In contrast the wheat-canola meal diets had significant quadratic regressions for both ileal ($P = .050$) and fecal digesta ($P = .002$) but their significance was lower than that of the wheat-soybean meal fecal regression.

With quadratic regressions the CAAD values derived are no longer independent of AA intake because the derivatives of the quadratic regression contain a variable (Table V.3) which is the level of AA contained in the diet. To emphasize the influence that the percentage dietary

AA has on CAAD, the derivatives of the total AA quadratic regressions presented in Table V.3 were used to calculate the ileal and fecal CAAD at each level of AA in the experimental diets (Table V.4). When total CAAD were calculated from the derivative equations it was again apparent that the values were affected by level of AA intake, except for the ileal digestibilities obtained on the diets containing wheat-soybean meal. As the level of AA in the diet increased, fecal CAAD values decreased on both the wheat-soybean meal and wheat-canola meal diets and the ileal digestibility values also decreased on the wheat-canola meal diets. Examination of the range of CAAD values obtained for the diets containing wheat-soybean meal or wheat-canola meal indicated that feeding a diet containing approximately 10% total AA would result in CAAD derived from the quadratic regressions similar to those from the linear regressions. For that reason the digestibilities derived from the linear regressions appeared to be as suitable as those derived using the quadratic regressions for comparing CAAD within and between the protein sources.

The apparent decrease in CAAD when the levels of AA contained in the diets were increased was an unexpected observation based on the previous experiments (Chapters 2 and 4). The observation was also in contrast to the reported effects of protein intake on CAAD in swine and poultry (Carlson and Bayley, 1970; Sibbald, 1979a). No explanation for the decrease in CAAD upon the increase in AA concentration in the diets was apparent from the present data.

In comparing the present experiment with that of Sibbald (1979a) some differences in the methodology were evident. Sibbald used mature Leghorn cockerels which were force-fed 6, 12, 18, 24, or 30 grams of soybean meal and found no difference in CAAD related to intake. In the present experiment the chicks had an average initial body weight of 720 grams, which was considerably less than the weight of mature cockerels The average dietary intake of the diets containing 91% test ingredients was 111.1 grams with an average protein content of 20%. Thus the chicks in this experiment had maximum protein intakes of approximately 22 grams in a 12 hr period; whereas, the cockerels had a maximum intake of approximatley 14 grams of soybean

meal protein in a single feeding. The question arises as to what controls protein digestion in poultry. Is the percentage protein or AA content in the diet the more important factor or is the total grams of protein entering the gastrointestinal tract per unit time more important? If the bird has a maximum capacity to digest and/or absorb protein per unit of time, then the maximum could conceivably be reached when high levels of protein are fed.

There is some indication that absorption of amino acids may be rate limiting. The absorption of serine, alanine and methionine has been shown to be dependent on the concentration present in the ileum of the rabbit (Paterson et al., 1979), but this and other similar experiments (Matthews, et al., 1979) involved single AA in in vitro experiments. In more practical studies conducted in vivo, AA are known to be absorbed as peptides as well as in the free AA form (Kan, 1975). In addition dietary AA sources are also known to be diluted 1.5 to 4 times by endogenous AA sources (Bird, 1969; Crompton and Nesheim, 1968). Given this large dilution of dietary AA by endogenous AA, the absorption of AA and peptides from the digestion of dietary proteins would unlikely to be the rate limiting step. In a review (Snook, 1974) it was concluded that "hydrolysis is usually the rate limiting step in the release and absorption of AA". This does not provide conclusive evidence that digestion rather than the absorption process is a rate limiting step, but tends to suggest that digestion is the more important factor. In the present experiment the smaller birds consuming roughly equivalent amounts of protein may have reached a saturation point in the capacity of the digestive tract to digest the dietary protein.

To gain a further insight into the reasons for the differences between ileal and fecal CAAD, the individual and total AA excreted from the ileum and in the feces were compared. To do this the grams AA excreted from the ileum and in the feces per 100 grams feed intake were regressed on each other to obtain linear regressions (Table V.5). The results obtained indicated that there was a slope or ratio of 1.02 between the total grams ileal AA versus total grams fecal AA per 100 grams feed intake for the wheat-soybean meal diets and the corresponding ratio for the diets containing wheat-canola meal was 1.64.

A linear regression with a slope or ratio greater then one between the grams of ileal AA excreted versus fecal AA excreted would indicate that a lesser amount of AA was excreted in the feces in proportion to the amount excreted from the terminal ileum. This would suggest a net loss of AA between the terminal ileum and the feces as the excretion of AA from the terminal ileum increased. Sauer et al. (1977) and Low (1979) have demonstrated in pigs that AA can be lost in the hindgut through deamination. Canola meal being a less digestible protein source than soybean meal would have a greater excretion of AA into the hindgut and therefore potentially more AA available for deamination. The net effect on CAAD is that the fecal digestibilities will be greater than the corresponding ileal digestibilities. The higher the ratio is above 1.0 the greater the difference will be between ileal and fecal CAAD. The data presented in Tables V.2 and 5 support this observation. The ratio between the total ileal and fecal grams AA excreted on the wheat-soybean meal diets was 1.02 (Table V.5) and the total ileal and fecal CAAD were 89.2% and 94.9%, respectively (Table V.2). In comparison on the wheat-canola meal diet the ileal-fecal ratio was 1.64 for the total AA and the corresponding CAAD were 81.4% and 90.4%, respectively, for the ileal and fecal digesta. This indicated that there was a greater change in the AA profile in the hindgut digesta of birds fed the wheat-canola meal diet than in the digesta of those fed the wheat-soybean meal diet

The influence of diet and the effects of fasting and feeding periods on dietary intake are shown in Table V.6. Lower intakes occurred on the N-free diets but otherwise diet did not significantly affect feed intake. Increasing the fasting period to 24 or 36 hr had no effect on subsequent feed intake during the 12 hr feeding period. When the feeding period after a 12 hr fast was extended to 24, 48 or 72 hr feed intake increased in relation to the time the birds were given to feed.

The influence of length of the fasting period and length of the feeding period on the levels of AA excreted are presented in Table V.7. Increasing the length of starvation from 12 to 24 hr, significantly increased the concentration of AA found in the ileal digesta after feeding the N-free diets. A further increase in the length of starvation from 24 to 36 hr did not cause a

further increase in the AA concentration of the ileal digesta. The concentration of AA in the fecal digesta collected were not affected by fasting length. Feeding periods of 12, 24, 48 or 72 hr had no effect on the concentration of AA found in the ileal or fecal digesta.

D. Summary

The wheat-soybean meal and wheat-canola meal diets had lower individual and total, ileal CAAD than fecal CAAD. The differences for total CAAD was less for the wheat-soybean meal diets (89.2% versus 94.9%) than for the wheat-canola meal diets (81.4% versus 90.4%). The regressions of digested AA on AA intake showed a significant curvilinear response, which resulted in a decrease in estimated CAAD as AA intake increased. The length of fasting or feeding period did not affect the concentration of AA found in the ileal or fecal digesta.

Table V.1 Composition of the experimental diets.

Ingredients %	Diet No. 1	2	3	4	5	6	7
Corn starch	91	61	30	-	61	30	-
Ground wheat	-	22	45	67	11	23	34
Soybean meal	-	8	16	24	-	-	-
Canola meal	-	-	-	-	19	38	57
Constant ingredients[1]	9	9	9	9	9	9	9

[1]Supplied the following levels per kg of diets: corn oil 31g; ground limestone 8g; Biofos (18% Ca-21% P) 33g; iodized salt 6g; chromic oxide 1 g; vitamin-mineral premix 11g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D_3 600 ICU; vitamin E 10 IU; menadione 3mg; riboflavin 6mg; calcium pantothenate 10mg; niacin 15mg; folic acid 1mg; biotin .2mg; vitamin B_{12} 10mcg; choline chloride 1500mg; manganese 100mg; zinc 80mg.

Table V.2 Ileal and fecal amino acid digestibility of tested protein sources obtained by linear regression.

Amino acid	Wheat - Soybean meal		Wheat - Canola meal	
	Ileal Digestibility %	Fecal Digestibility %	Ileal Digestibility %	Fecal Digestibility %
Aspartic acid	86.2 ± 1.1[1]	91.0 ± 1.7	73.3 ± 1.4	84.6 ± 1.5
Threonine	87.3 ± 1.7	96.8 ± 2.6	74.1 ± 1.7	88.6 ± 1.7
Serine	88.8 ± 1.2	96.6 ± 2.0	77.9 ± 1.3	90.0 ± 1.8
Glutamic acid	93.5 ± .6	96.1 ± .8	88.2 ± .8	93.9 ± .8
Proline	90.2 ± 1.6	94.6 ± 2.2	79.7 ± 1.2	86.8 ± 2.5
Glycine	83.8 ± 1.5	-	76.0 ± 1.4	-
Alanine	87.0 ± 1.4	92.4 ± 1.7	83.7 ± .8	89.9 ± 1.4
Valine	83.6 ± 2.2	91.9 ± 2.5	74.2 ± 2.1	87.5 ± 1.9
Methionine	86.2 ± 2.5	90.6 ± 2.8	87.1 ± 1.0	92.3 ± 2.0
Isoleucine	87.6 ± 1.4	94.2 ± 1.5	80.6 ± 1.1	90.5 ± 1.3
Leucine	88.6 ± 1.1	94.9 ± 1.2	83.0 ± .9	92.5 ± 1.0
Tyrosine	80.5 ± 4.6	105.0 ± 5.2	70.8 ± 5.8	97.0 ± 4.4
Phenylalanine	88.9 ± 1.3	94.6 ± 2.1	82.2 ± 1.3	92.7 ± 2.4
Lysine	89.1 ± 1.5	95.0 ± 1.7	80.9 ± 1.0	89.7 ± 1.1
Histidine	89.0 ± 1.6	93.5 ± 2.3	84.8 ± 2.1	91.2 ± 2.4
Arginine	92.1 ± 1.8	96.3 ± 1.3	87.5 ± 1.0	93.8 ± .9
Total	89.2 ± 1.2	94.9 ± 1.5	81.4 ± 1.1	90.4 ± 1.3

[1]Digestibility coefficient ± standard error.

TABLE V.3. Quadratic regressions of grams total amino acid digested and grams total amino acid intake per 100 grams feed intake for ileal and fecal digesta and their derivatives.

Analysis	b_0	b_1x	b_1x^2	F† (probability)
Wheat - Soybean meal				
Ileal	-.685 ± .164‡	.896 ± .045	-.0002 ± .002	.01 (.923)
Derivative		.896 - .0004x		
Fecal	-2.026 ± .115	1.110 ± .031	-.008 ± .002	28.3 (.0002)
Derivative		1.110 - .016x		
Wheat - Canola meal				
Ileal	-.605 ± .164	.885 ± .034	-.003 ± .001	4.81 (.050)
Derivative		.885 - .006x		
Fecal	-2.046 ± .152	1.028 ± .033	-.005 ± .001	15.5 (.002)
Derivative		1.028 - .010x		

†Regression coefficients ± standard error.

‡F-value and probability of quadratic regression coefficient (B^2) equaling zero.

Table V.4 Comparison of total amino acid digestibilities derived from the quadratic regressions with values from the linear regressions.

Diet	Treatment	Amino acid content %	Digestibility Ileal %	Digestibility Fecal %
Quadratic			Wheat-Soybean meal	
1	N-free	0	89.6	111.0
2	30% W-SBM[1]	6.3	89.3	101.0
3	61% W-SBM	12.7	89.1	90.7
4	91% W-SBM	18.9	88.8	80.7
Linear		-	89.2	94.9
Quadratic			Wheat-Canola meal	
1	N-free	0	88.5	102.8
5	30% W-CM[2]	8.1	83.7	94.7
6	61% W-CM	15.7	79.1	87.1
7	91% W-CM	23.7	74.3	79.1
Linear		-	81.4	90.4

[1]Wheat-Soybean meal

[2]Wheat-Canola meal

Table V.5 The slopes of the linear regressions used to compare the levels of ileal and fecal amino acids.

Amino acid	Wheat - Soybean meal slope gram/gram	Wheat - Canola meal slope gram/gram
Aspartic acid	1.14 ± .20[1]	1.56 ± .15
Threonine	.67 ± .35	1.84 ± .21
Serine	.80 ± .37	1.68 ± .22
Glutamic acid	1.09 ± .22	1.65 ± .16
Proline	.65 ± .27	1.08 ± .21
Alanine	1.14 ± .22	1.37 ± .16
Valine	.98 ± .31	1.62 ± .25
Methionine	.71 ± .25	.92 ± .25
Isoleucine	1.08 ± .28	1.60 ± .22
Leucine	1.20 ± .28	1.87 ± .20
Tyrosine	.17 ± .28	.59 ± .56
Phenylalanine	.77 ± .27	1.18 ± .33
Lysine	.98 ± .29	1.53 ± .14
Histidine	.59 ± .19	.96 ± .25
Arginine	.75 ± .40	1.72 ± .19
Total	1.02 ± .30	1.64 ± .20

[1]Slope coefficient ± standard error.

Table V.6 Influence of dietary treatment and intervals of fasting and feeding on feed intake.

Diet	Treatment	Fasting and feeding interval	Avg. feed intake/chick in a pen
1	N-free	12hr fast; 12hr feed	75.2 ± 1.4
2	30% wheat-soybean meal	"	110.4 ± 9,0
3	61% wheat-soybean meal	"	123.9 ± 9.7
4	91% wheat-soybean meal	"	115.8 ± 4.8
5	30% wheat-canola meal	"	109.8 ± 14.1
6	61% wheat-canola meal	"	112.0 ± 17.0
7	91% wheat-canola meal	"	106.4 ± 8.3
4	91% wheat-soybean meal	12hr fast; 24hr feed	172.8 ± 3.0
4	91% wheat-soybean meal	12hr fast; 48hr feed	309.3 ± 20.9
4	91% wheat-soybean meal	12hr fast; 72hr feed	362.5 ± 1.4
1	N-free	12hr fast; 24hr feed	88.4 ± 1.6
1	N-free	12hr fast; 48hr feed	169.8 ± 2.6
1	N-free	24hr fast; 12hr feed	99.9 ± 2.7
1	N-free	36hr fast; 12hr feed	97.1 ± 3.1

Table V.7 The influence of fasting period and time of collection after feeding on the total grams amino acid excreted per 100 grams feed intake.

Diet	Treatment	Fasting Period 12hr	24hr	36hr	
1	Ileal N-free	.59 ± .10*a*	1.17 ± .26*b*	1.16 ± .52*b*	
1	Fecal N-free	1.97 ± .20	2.01 ± .34	2.14 ± 54	
		Time of feeding 12hr	24hr	48hr	72hr
1	Ileal N-free	.59 ± .10	.66 ± .14	.72 ± .10	.58 ± .15
4	Ileal 91% W-SBM[1]	2.47 ± .17	2.31 ± .09	2.34 ± .03	2.46 ± .07
4	Fecal 91% W-SBM	2.93 ± .19	3.02 ± .05	3.45 ± .37	2.87 ± .20
7	Ileal 91% W-CM[2]	4.66 ± .35			4.56 ± .15
7	Fecal 91% W-CM	4.33 ± .30			4.28 ± .04

a,b Means of dietary treatments followed by different subscripts are significantly different $P < .05$.

[1]Wheat-Soybean meal

[2]Wheat-Canola meal

VI. Ileal and Fecal Amino Acid Digestibility Estimates of Soybean Meal and Canola Meal using Broiler Chicks.

A. Introduction

In conjunction with the experiment in Chapter 5 an experiment was conducted to estimate the corrected amino acid digestibility (CAAD) of soybean meal and canola meal. If passing through the hindgut of poultry has no influence on the profile of the amino acid (AA) in the digesta then the estimates of CAAD derived using the ileal digesta should not be significantly different from the estimates derived using the fecal (excreta) material. Soybean meal and canola meal provide protein sources with two different digestibilities (Muztar et al., 1980). In pigs and chicks less digestible protein sources have been shown to undergo greater alteration of their AA profile when passing through the hindgut (Holmes et al., 1974; Varnish and Carpenter, 1975).

In the present study the change in AA composition while passing through the hindgut of poultry and its effect on CAAD was evaluated again using protein sources of two different digestibilities.

B. Materials and Methods

The breed of chickens used, their management and the procedure followed in the collection of the ileal digesta in this experiment were similar to those described in Chapter 4. The only difference was the chicks received continuous lighting and were housed four chicks per cage and during the three days of digesta collections a pen of four chicks per diet were sacrificed on three consecutive days. The digesta from the four chicks per pen sacrificed each day were pooled for analysis.

Three pens of chicks were assigned to each of the seven experimental diets (Table VI.1). The experimental diets contained graded levels of soybean meal or canola meal and a diet without added protein containing 86% autoclaved corn starch served as a N-free diet.

Cellulose was added at a 5% level to all of the diets in order to increase the amount of digesta available for collection from the ileum. Chromic oxide was added at 0.1% to all diets to serve as an inert marker.

The laboratory and statistical analysis conducted were similar to those described in Chapter 4. The only difference in this experiment was that the AA analyses of the digesta samples were not done in duplicate because of the small amount of variation in previous experiments in which variation between duplicates was not significant as compared to the variation between replicates.

C. Results and Discussion

The CAAD derived using the derivatives of the linear regressions of grams AA digested versus grams AA intake are presented in Table VI.2. The intercepts of these linear regressions are contained in Appendix XII.8. The quadratic regressions for the total AA and their derivatives are presented in Table V.3. The quadratic regressions of individual AA are contained in Appendices XII.9, 10, 11, and 12. Soybean meal had only a few AA which did not have a significantly better fit with the quadratic regressions than with the linear regressions for both the ileal and fecal digesta. Canola meal however was different in that the ileal digesta had significant quadratic regressions for all of the AA but the fecal digesta had non-significant quadratic regressions for all of the AA.

As indicated in Chapter 5, with a quadratic regression the percentage AA in the diet influences the digestibility value estimated, and therefore no single value for CAAD can be obtained. The grams AA intake per 100 grams dietary intake and the resulting grams AA excreted in the digesta are presented in Table VI.4. In Table VI.5 the AA intakes represented by the experimental diets were substituted into the derivatives of the total AA quadratic regressions (Table VI.3) to obtain total CAAD. From the range of the total CAAD for soybean meal and canola meal it can be seen that an approximate intake of 13% total AA or protein intake would give digestibilities similar to the linear derived digestibilities. Again, for

this reason the digestibilities derived using the linear derivatives were used to compare CAAD within and between protein sources. The ileal digestibility values (Table VI.2) were lower for canola meal (76.0%) than for soybean meal (83.0%), whereas the fecal digestibilities were similar 85.8% and 87.4% for canola meal and soybean meal respectively. The quadratic constant (b_2) in the ileal regressions were larger than the quadratic constant in the fecal regressions. As a consequence the ileal CAAD were more sensitive to the percentage dietary AA intake than the fecal CAAD.

The CAAD derived from linear regressions were compared to other values reported in the literature (Tao et al., 1971; Nwokolo et al., 1976; Achinewhu and Hewitt, 1979; Sibbald, 1979a; Muztar et al., 1980; and Parsons et al., 1981; and Engster 1983). The AA digestibility values of soybean meal are contained in Table VI.6 and those of canola meal are contained in Table VI.7. Since the only extended list of AA digestibility values were derived using fecal digesta only the fecal AA digestibility were used for comparison. In the present experiment the total CAAD was calculated for each of the protein sources, however, in the literature total CAAD was not calculated in all cases, therefore average AA digestibility was used as a reference for comparing the data.

For both soybean meal and canola meal the absolute values of the average AA digestibility varied considerably which is probably a reflection of the different methods used to obtain them. The range of the individual AA digestibility from the average AA digestibility showed some similarities between the experiments. In soybean meal the digestibility of threonine and alanine were lower than average AA digestibility, while the digestibilities of glutamic acid and phenyalanine were higher than average. Canola meal also had particular AA with digestibilities higher or lower than average AA digestibility. Threonine, serine and proline had digestibilities that were lower average AA digestibility and glutamic acid, phenyalanine and arginine had higher than average digestibilities. If particular AA continually have higher or lower than average AA digestibility, then the use of a nitrogen or protein digestibility could be more useful in predicting the digestibility of individual AA in feedstuffs.

Researchers evaluating the effect of hindgut microflora and urine amino acid contamination on fecal AA have used different chickens for their comparisons and have also only assessed the effect of either the microflora or urine AA contamination (Bragg et al., 1969; Salter and Fulford, 1974; Varnish and Carpenter, 1975). In the present experiment the same pen of chickens were used to compare the excretion of ileal and fecal grams AA per 100 grams feed intake, thus removing animal variation. To achieve a comparison between ileal and fecal AA profiles, the ileal grams AA excretion per 100 grams feed intake was regressed on the fecal grams AA excretion per 100 grams feed intake (Table VI.7). The resulting slope or ratio between the ileal and fecal AA excretion provided a basis on which to establish whether AA were gained or lost in the hindgut. The quadratic regressions did not give a significantly better fit than the linear regression and therefore only the linear regressions are presented. If there were no alteration of AA in the hindgut the ratio between the increase in ileal and fecal grams AA excreted per 100 grams feed intake should be one. As shown in Table VI.7 the ratio was 1.28 for soybean meal and 1.45 for canola meal. A ratio greater than one indicates that there was a loss of AA as the digesta passed through the hindgut. Consequently calculated ileal digestibilities were lower than the fecal digestibilities.

The results demonstrate that the derived CAAD depend on whether ileal or fecal digesta were used for the calculations (Tables VI.2, 3 and 4). The difference between ileal and fecal derived CAAD increased as the ratio between ileal and fecal AA became larger. Canola meal had a larger ratio between the ileal and fecal total AA than soybean meal, and the difference between the ileal and fecal total CAAD was also larger (Table VI.2). The same pattern of results was observed in Chapter 4. The diets containing canola meal had a larger ratio (1.6) between ileal and fecal total AA as compared to a ratio of 1.0 for the diets containing soybean meal. The resulting estimates of ileal and fecal CAAD were 81.4% and 90.4%, respectively, for diets containing canola meal and 89.2% and 94.9%, respectively, for diets containing soybean meal.

A possible reason for the loss of AA in the hindgut may be the deamination of the AA by microflora. Work with pigs and chicks has shown AA entering the hindgut can be deaminated by the microflora, especially when an energy source for the microflora is limiting (Nesheim and Carpenter, 1969; Holmes et al., 1974; Sauer et al. 1977; Just et al., 1981) Canola meal being a less digestible protein source than soybean meal had more residual AA entering the hindgut and therefore a greater potential source of AA for microbial deamination. This may have been responsible for the greater decrease in the level of AA while passing through the hindgut.

There was considerable variability in the digestibility of tyrosine and methionine in the present study and in the others cited. This may have been caused by oxidation of these AA during hydrolysis. Tyrosine is susceptible to oxidation by traces of residual oxygen present during hydrolysis and therefore its recovery can be variable (Kohler and Palter, 1967). Methionine may also be oxidised in varying amounts to methionine sulphone depending the amount of carbohydrate present during hydrolysis in 6 N HCl. For this reason in order to measure methionine quantitatively, performic acid should be used before hydrolysis to convert all methionine to methionine sulphone and then analysis should be conducted for the methionine sulphone (Lewis, 1966). Because performic acid was not used, the methionine digestibilities in the present studies may not be entirely reliable.

The intercept values of the quadratic regressions are estimates of endogenous AA excretions at zero protein intake. The estimates of the total grams AA excreted from the ileum per 100 grams intake of a N-free diet were .63 - .65 (Table VI.3). The corresponding estimates for fecal digesta were 1.23 - 1. 24 grams. These values point out the contribution of AA from urine, hindgut cell slough and microflora is approximately twice that found at the terminal ileum. To a large degree it is this difference between the excretion of AA from the ileum and the hindgut which causes the difference between the ileal and fecal CAAD.

D. Summary

The CAAD of soybean meal and canola meal was affected by the hindgut of poultry, the effect being greatest with canola meal. Both the ileal and fecal digestibility estimates had a curvilinear response, with a decrease in CAAD as AA intake increased. This effect was significant for the total CAAD for the ileal and fecal digesta collections of soybean meal, and only significant for the ileal digesta collections for canola meal. In comparison to other reported AA digestibility values the absolute values varied, however, the pattern of digestibility of individual AA in comparison to average AA digestibility was relatively uniform.

Table VI.1 Composition of experimental diets.

Ingredients %	Diet No. 1	2	3	4	5	6	7
Corn starch	86	64	42	20	58	29	-
Soybean meal	-	22	44	66	-	-	-
Canola meal	-	-	-	-	28	57	86
Constant ingredients[1]	14	14	14	14	14	14	14

[1]Supplied the following levels per kg of diets: cellulose 50g[2], corn oil 31g; ground limestone 8g; Biofos (18% Ca-21% P) 33g; iodized salt 6g; chromic oxide 1 g; vitamin-mineral premix 11g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D_3 600 ICU; vitamin E 10 IU; menadione 3mg; riboflavin 6mg; calcium pantothenate 10mg; niacin 15mg; folic acid 1mg; biotin .2mg; vitamin B_{12} 10mcg; choline chloride 1500mg; manganese 100mg; zinc 80mg.

[2]Alphafloc; Brown Company, Berlin, New Hampshire.

Table VI.2 Ileal and fecal amino acid digestibility of soybean meal and canola meal obtained by using the linear regressions.

Amino acid	Soybean meal		Canola meal	
	Ileal Digestibility %	Fecal Digestibility %	Ileal Digestibility %	Fecal Digestibility %
Aspartic acid	79.2 ± 2.2[1]	85.3 ± 1.4	67.7 ± 2.4	80.3 ± 2.3
Threonine	79.3 ± 2.3	86.4 ± 2.1	67.0 ± 2.1	80.3 ± 2.3
Serine	81.6 ± 1.8	87.8 ± 1.4	68.7 ± 2.6	81.1± 2.1
Glutamic acid	85.7 ± 1.7	88.7 ± 1.2	83.8 ± 1.6	90.0 ± 1.3
Proline	72.6 ± 3.1	83.5 ± 2.9	66.7 ± 2.3	80.6 ± 2.8
Glycine	78.5 ± 2.4	-	66.7 ± 3.0	-
Alanine	84.7 ± 1.9	87.6 ± 1.3	78.9 ± 2.1	87.5 ± 1.9
Valine	79.3 ± 3.0	83.0 ± 2.2	70.2 ± 3.2	84.1 ± 2.7
Methionine	77.3 ± 4.4	83.6 ± 3.1	81.3 ± 2.5	91.8 ± 2.1
Isoleucine	81.7 ± 2.1	86.4 ± 1.6	72.8 ± 2.4	86.6 ± 2.3
Leucine	82.8 ± 1.8	87.5 ± 1.5	77.9 ± 2.2	87.9 ± 1.7
Tyrosine	80.8 ± 2.3	87.3 ± 1.9	67.0 ± 3.2	83.4 ± 2.9
Phenylalanine	83.9 ± 2.0	87.9 ± 1.7	78.0 ± 2.6	89.1 ± 2.2
Lysine	88.6 ± 1.0	89.3 ± 1.1	77.9 ± 1.9	83.5 ± 1.6
Histidine	86.9 ± 2.0	90.0 ± 1.6	82.5 ± 1.5	88.6 ± 1.2
Arginine	86.8 ± 1.7	89.3 ± 1.22	85.7 ± 1.2	90.0 ± 1.0
Total	83.0 ± 1.8	87.4 ± 1.4	76.0 ± 2.0	85.8 ± 1.8

[1]Digestibility coefficient ± standard error.

TABLE VI.3. Quadratic regressions and their derivatives of grams total amino acid digested versus grams total amino acid intake per 100 grams feed intake.

Analysis	b_0	b_1x	b_2x^2	F† (probability)
Soybean meal				
Ileal	-.651 ± .198‡	.989 ± .036	-.006 ± .001	21.0 (.001)
Derivative		.989 - .012x		
Fecal	-1.237 ± .191	.978 ± .035	-.004 ± .001	9.6 (.013)
Derivative		.978 - .008x		
Canola meal				
Ileal	-.630 ± .140	.956 ± .024	-.007 ± .001	71.4 (.0001)
Derivative		.956 - .014x		
Fecal	-1.229 ± .376	.907 ± .065	-.002 ± .002	.6 (.451)
Derivative		.907 - .004x		

†F-value and probability of quadratic regression coefficient (B_2) equaling zero.

‡Regression coefficients ± standard error.

Table VI.4 Total amino acid content in the ileal and fecal digesta of chicks fed diets containing soybean meal or canola meal.

Diet	Treatment	Amino acid content		
		Dietary	Ileal	Fecal
		g	g	g
1	N-free	-	.61 ± .03	1.22 ± .31
2	22% SBM[1]	8.79	1.32 ± .07	1.76 ± .26
3	44% SBM	17.57	2.61 ± .23	2.82 ± .54
4	66% SBM	26.36	5.17 ± .68	4.61 ± .28
5	28% CM[2]	9.33	1.69 ± .14	2.26 ± .77
6	57% CM	18.33	3.78 ± .28	3.54 ± .70
7	86% CM	27.66	7.29 ± .43	5.16 ± .89

[1]Soybean meal

[2]Canola meal

Table VI.5 Comparison of total amino acid digestibilities derived from the quadratic regressions with values from the linear regressions.

Diet	Treatment	Amino acid content %	Digestibility Ileal %	Digestibility Fecal %
Quadratic		Soybean meal		
1	N-free	0	-	-
2	22% SBM[1]	8.8	88.3	90.8
3	44% SBM	17.6	77.7	83.9
4	66% SBM	26.4	67.1	77.0
Linear		-	83.0	87.4
Quadratic		Canola Meal		
1	N-free	0	-	-
5	28% CM[2]	9.3	82.4	87.4
6	57% CM	18.3	69.6	84.2
7	86% CM	27.7	56.2	80.9
Linear		-	76.0	85.8

[1]Soybean meal

[2]Canola meal

Table VI.6 A comparison of fecal amino acid digestibility values of soybean meal reported in the literature.

Amino acid	Achinewhu[1] %	Sibbald[2] %	Muztar[3] %	Parsons[4] %	Engster[5] %	Present Studies %
Aspartic acid	92	92.0	93	92.1	92.0	85.8
Threonine	91	90.5	91	87.3	88.2	86.4
Serine	93	92.6	88	91.0	90.0	87.8
Glutamic acid	94	-	94	94.2	93.6	88.7
Proline	91	94.3	90	91.3	91.1	83.5
Alanine	93	90.2	90	88.6	87.6	87.6
Valine	96	92.3	91	90.9	90.3	83.0
Methionine	95	-	83	94.2	93.2	83.6
Isoleucine	94	91.7	94	91.6	91.9	86.4
Leucine	93	91.9	91	91.3	91.7	87.5
Tyrosine	92	94.4	84	94.8	94.4	87.3
Phenylalanine	94	94.0	96	91.5	93.6	87.9
Lysine	97	97.0	93	87.6	91.7	89.3
Histidine	91	93.2	85	91.4	90.4	90.0
Arginine	92	92.0	93	92.1	92.0	89.3
Average	93.4	92.7	90.3	91.2	91.5	86.9

[1]Achinewhu and Hewitt, 1979.

[2]Sibbald, 1979.

[3]Muztar et al., 1980.

[4]Parsons et al., 1981.

[5]Engster et al., 1983.

Table VI.7 A comparison of fecal amino acid digestibility values of canola meal reported in the literature.

Amino Acid %	Tao[1] %	Nwokolo[2] %	Muztar[3] Tower %	Muztar[3] Candle %	Present Studies %
Aspartic acid	62.8	91.7	80	74	80.3
Threonine	63.5	90.8	74	67	80.3
Serine	70.1	91.4	78	68	81.1
Glutamic acid	78.8	94.9	92	85	90.0
Proline	81.9	91.2	54	68	80.6
Alanine	65.8	94.2	85	77	87.5
Valine	59.8	90.9	81	74	84.1
Methionine	74.8	78.4	86	79	91.8
Isoleucine	64.8	91.6	81	74	86.6
Leucine	71.6	94.0	87	83	87.9
Tyrosine	63.6	92.8	89	79	83.4
Phenylalanine	69.3	94.8	87	83	89.1
Lysine	72.8	94.4	80	73	83.5
Histidine	71.6	94.2	83	80	88.6
Arginine	75.7	95.8	88	82	90.0
Average	69.8	92.0	81.7	76.4	85.6

[1]Tao, et al., 1971.

[2]Nwokolo et al., 1976.

[3]Muztar et al., 1980.

Table VI.8 The slopes of the linear regressions of ileal versus fecal grams amino acid found in the digesta per 100 grams feed intake.

Amino acid	Soybean meal Slope grams/gram	Canola meal Slope grams/gram
Aspartic acid	1.39 ± .10[1]	1.47 ± .18
Threonine	1.39 ± .13	1.52 ± .16
Serine	1.48 ± .12	1.56 ± .17
Glutamic acid	1.23 ± .10	1.50 ± .15
Proline	1.30 ± .27	1.39 ± .25
Alanine	1.16 ± .14	1.42 ± .22
Valine	1.17 ± .16	1.51 ± .25
Methionine	.96 ± .28	1.33 ± .39
Isoleucine	1.22 ± .17	1.57 ± .24
Leucine	1.32 ± .11	1.67 ± .19
Tyrosine	1.33 ± .18	1.51 ± .32
Phenylalanine	1.18 ± .16	1.47 ± .32
Lysine	1.01 ± .10	1.25 ± .15
Histidine	1.10 ± .23	1.47 ± .12
Arginine	1.12 ± .20	1.40 ± .12
Total	1.28 ± .11	1.45 ± .81

[1]Slope ± standard error.

VII. Estimates of Ileal and Fecal Amino Acid Digestibility of Soybean Meal and Dried Egg Albumen in Broiler Chicks.

A. Introduction

In previous experiments (Chapters 5 & 6) it became evident that the method used to estimate corrected amino acid digestibility (CAAD) often results in a curvilinear relationship between amino acid (AA) intake and CAAD. As AA intake increases the estimated CAAD decreases. This finding is in conflict with other reports on the effects of increased AA intake on AA digestibility (Carlson and Bayley, 1970; Sibbald, 1979a), which indicated that there was a linear relationship between AA intake and its digestibility.

The curvilinear response in CAAD results in different estimates of digestibility as AA intake changes. This causes problems in utilizing CAAD values in the formulation of practical poultry diets. Most of the other methods used in estimating CAAD used total fecal collections whereas in the present studies collections of samples of digesta containing a marker have been used to estimate CAAD. It therefore seemed desirable that a further study be conducted to determine whether the response in CAAD would be affected by collection method. A trial was designed to determine if the method of digesta collection would affect the curvilinear response in CAAD. A second trial was conducted to see if a highly digestible protein source, such as egg albumen, would also show the same curvilinear response in CAAD as found with other protein sources.

B. Material and Methods

The breed of chickens used and their management to four weeks of age were the same as outlined in Chapter 4, except the chicks were provided with continuous lighting. In the first trial the diets (Table VII.1) consisted of four levels of soybean meal plus a N-free diet containing mainly autoclaved corn starch. Soybean meal was added to the diets at levels of 0, 22, 44, 66, or 88% replacing autoclaved corn starch. Cellulose was added at decreasing levels

with increasing levels of soybean meal in order to keep crude fiber levels comparable. In the second trial dried egg albumen was added to the diets at levels of 0, 10, 20, 30, or 40% replacing autoclaved corn starch. Cellulose was added at a level of 5% to all diets as a source of fiber.

In each trial four chicks were housed per cage and three groups of chicks were placed on each of the five experimental diets. In the first trial, for total fecal collections, the chicks were fasted for 12 hr, fed the experimental diets for 48 hr, and then fasted again for the final 12 hr. The total excreta produced were collected 24, 48 and 60 hr after the diets were introduced and were immediately frozen. To obtain the samples of fecal (excreta) material and ileal digesta the final 12 hr fast period following the total fecal collections served as the intial 12 hr fast period for sample collections. After the 12 hr fast the chicks were refed the experimental diets for 12 to 13 hr with the final 2 hr of the feeding period being used to collect the sample fecal material. At the end of the 12 hr feeding period the chicks were sacrificed during the following hour, a pen at a time, and the ileal digesta collected. In the second trial the chicks were fasted for 12 hr before being fed the experimental diets for 12 to 13 hr. At the end of the 12 hr feeding period the chicks were sacrificed over an one hour period and the ileal digesta collected as outlined in Chapter 4. All chicks were sacrificed on the same day. The digesta from the individual chicks within a pen were pooled for laboratory analysis as outlined in Chapter 4.

For the data analysis of the two trials, the grams AA excreted were regressed on grams AA intake. This was different from the previous analysis in which AA digested was regressed on AA intake. The linear, quadratic and logarithmic regressions were computed for each collection method. The regressions were used to obtain the amount of AA excreted given the AA intake. Using the following equation CAAD could then be predicted from the regressions at the chosen AA intakes.

Digestibility = (Intake - Excretion)/Intake

At a given AA intake the CAAD can be determined either for that last increment of added AA or for the combined digestibility of the different levels of AA from zero to whatever intake is chosen. The derivative of the regression at a given level of AA intake and the integral

of the regression are used to obtain the combined CAAD up to a given level of intake. The integral of the regression may then be used to obtain the area under the curve representing the excreted AA. The excreted AA contains AA of both dietary and endogenous sources, therefore before the CAAD can be derived the portion represented by endogenous AA has to be subtracted from the total of the excreted AA. An assumption was made that the correction for endogenous AA excretions was equal to the area underneath the line created by the intercept at zero AA intake (Figure VII.1).

The coefficient of variation (Steel and Torrie, 1980) was used to determine whether the levels of AA excreted were more of dietary origin or endogenous origin. If the levels of AA excreted were more of dietary origin then it was postulated the pattern would resemble the AA pattern of the protein source, conversely if the pattern of AA excreted were more of endogenous origin then it was assumed the pattern would resemble the AA pattern excreted on the N-free diet The AA levels of the dietary protein source and of the endogenous AA were divided by the AA levels excreted when the birds were fed the protein source to obtain two sets of ratios. The coefficient of variation was used to determine which set of ratios derived had the least variation from the mean and therefore the greatest similarity to the AA levels excreted when the protein source was fed.

C. Results and Discussion

In the first trial the linear, quadratic and logarithmic regressions of total excreted AA versus total feed AA intake for the three collection methods for soybean meal were derived (Table VII.2). The linear, quadratic and logarithmic regressions for the individual AA are presented in Appendices XII.13, 14, 15, 16 and 17. With each method of sample collection all of the quadratic regressions had significantly better fits than the corresponding linear regressions. In contrast when the total fecal collections were used only four of the AA, aspartic acid, glutamic acid, leucine and arginine displayed a significantly better fit with the quadratic regressions. The total AA for the logarithmic regressions had correlations for the ileal and fecal

samples collections similar to those of the quadratic regressions. The total fecal collections had better correlations with the quadratic regressions than with the logarithmic regressions. The logarithmic regressions had exponential slopes for ileal and fecal samples collections which were similar for most of the AA, demonstrating that soybean meal had only small differences between ileal and fecal estimates of CAAD.

In Chapters 5 and 6 only the derivatives of the quadratic regressions were used to obtain the CAAD at different levels of AA intake. The CAAD obtained using the derivatives of the regressions do not take in to account the combined digestibility of the AA over the range from zero AA intake to the chosen level of AA intake. Mathematically the combined CAAD can be obtained using the integral of the regression. In Table VII.3 estimates of CAAD derived using the derivatives and integrals of the quadratic and logarithmic regressions are presented. The range of AA intakes chosen to use in deriving the regressions were the AA intakes represented by the experimental diets at 100 grams dietary intake.

The quadratic and logarithmic regressions gave similar estimates of CAAD for the ileal sample collections. The sample fecal and total fecal collections resulted in different estimates of CAAD between the two regression methods. The sample fecal collections had higher estimates of total endogenous AA excretion at zero protein intake using the quadratic regression than with the logarithmic regression, 1.4 versus 1.1 grams. As a result the derived CAAD was also higher for the quadratic regression (Table VII.3). The difference arose because of the assumption that the intercept at zero AA intake equals the endogenous AA output. There is no way of knowing which estimate of endogenous AA excretion is more accurate and therefore no way of explicitly stating which was the better estimate of CAAD. Errors in the curvilinear regressions may result if estimates of endogenous AA excretion are greater than the measured excretion at zero protein intake or if the quadratic regressions have initial slopes that are negative, such as was observed with in the sample fecal collection. A negative slope in a quadratic regression results in estimates of CAAD greater than 100% at low levels of AA intakes.

Logarithmic and quadratic regressions based on total fecal collections also showed differences between the estimates of CAAD. The quadratic regression was not significantly different from the linear regression. The logarithmic regression would fit the best exponential curve to the linear data resulting in an over-estimate of CAAD at lower levels of AA intake. Since most of the quadratic regressions applied to total fecal collections were not significantly different from the linear regressions, the best estimates of CAAD were provided by the linear regressions.

The results of this experiment and those reported in Chapters 5 and 6 demonstrate that curvilinear response resulting in a decrease in CAAD upon increased AA intake was not due to random experimental error. The reason for the curvilinear response was either due to an actual decrease in CAAD or to a possible systematic error in the methodology used to obtain the estimates. To gain a further understanding into why the CAAD of the sample ileal and fecal collections showed a curvilinear response to increased AA intake whereas the total fecal collections did not, the raw data values were compared. The estimated dry matter digestibilities of the sample collections were compared to the actual dry matter digestibilities of the total fecal collections (Table VII.4). When the dry matter digestibilities of the sample collections were obtained using the concentrations of the chromic oxide in the feeds divided by the concentrations in the digesta, there were no major differences in dry matter digestibilities whether estimated by the ileal and fecal sample collections or by total fecal collections. It therefore appears that the use of an inert marker to estimate dry matter digestibility did not cause sampling errors.

The percent total AA contained in the digesta of birds fed the soybean meal diets were compared on a dry matter basis to see if sampling errors as a result of digesta collection could have occurred (Table VII.5). As the percent soybean meal in the diets increased from 0% to 88%, the corresponding increase in the concentration of digesta AA increased from 3.5% to 14.1% in the ileal samples, 6.5% to 17.5% in the fecal samples, and from 8.2% to 12.5% in the total fecal collections. The increase in percent AA excretion as the protein content of the diet

increased was much less when total fecal collections were used than when ileal or fecal sample collections were used. It was this difference in percent AA excreted which was largely responsible for quadratic regressions based on total fecal collections not being significantly different from the linear regressions whereas those based on sample collections were significantly different. The increased levels of AA in the samples of ileal and fecal digesta correlated more closely with the increases in dietary AA than when total feces collections were used, suggesting that values based on ileal or fecal samples may have greater validity.

There was no indication that time of sampling had any influence on the curvilinear response observed with ileal and fecal sample collections. In preliminary experimental trials the concentration of chromic oxide was found to equilibrate by nine hours after feeding. In addition, in Chapter 5 sampling the digesta at 12, 24, 48 and 72 hr after initial feeding showed no significant influence on the concentration of AA found in the digesta. For these reasons the chromic oxide or AA concentration in the digesta should not have changed significantly 12 hr after feeding the experimental diets.

Differences between the CAAD values obtained with sample and total fecal collection methods may be influenced to some extent by several factors. Periodic sampling may not detect periodic differences in the fecal material; feed particles may be released at different times from the gizzard depending on the texture of the diet (Hill, 1971); and the periodic emptying of the cecal material may also be missed by sample collections (Duke, 1977). With total fecal collections, the feces stay on the trays for up to 24 hr before being frozen, whereas in the collection of fecal samples the feces are kept on the trays for a maximum of 2 hr before freezing. Thus during total fecal collection the possibility exists that the microflora may significantly alter the AA profile of the feces on the trays.

Another source of error that may lead to differences in estimates of CAAD is the possibility of an interaction between the levels of AA intake and the resulting AA excreted. In general, in the experiments conducted there were lower feed intakes on the N-free diets than on those containing protein. The cereal diets tended to have an increase in feed intake with

increasing levels of dietary protein, whereas when the diets with soybean meal were fed there was an increase in dietary intake to a certain level and then intake tended to level off. The canola meal diets were unusual in that there was a decrease in dietary intake as the concentration of canola meal in the diets increased. These variations in dietary intakes (Chapters 2 and 5, and Appendix XII.18) demonstrate that although the graded levels of the added protein source may influence dietary intakes the changes were not systematic and therefore would not be responsible for the curvilinear response in AA excretion observed upon increased AA intake. Other studies have indicated that carbohydrate source, especially fiber, may influence CAAD (Brown, et al., 1979; Okumura, et al., 1982). For this reason an attempt was made to keep the crude fiber levels of the diets constant.

In the second trial with the different levels of egg albumen in the diets, using only ileal collections, regressions between AA excretion and feed AA intake were derived. In this trial the quadratic regressions gave a significantly better fit than the linear regressions for all of the individual AA and total AA. For this reason the linear regressions are not presented. In Table VII.6 the total AA quadratic and logarithmic regressions are presented. The quadratic and logarithmic regressions for the individual AA of egg albumen are contained in Appendices XII.19 and 20. The CAAD of egg albumen was calculated in the same manner as that used with soybean meal. The results (Table VII.7) indicated that an error in the estimates of CAAD occurred with the quadratic regressions because of an over-correction for endogenous AA excretion. The quadratic regressions for total AA had a negative slope up to 6.0% total AA intake (Figure VII.1), thus causing the over-correction for endogenous AA excretion and an estimates of CAAD greater then 100% (Table VII.7). The logarithmic regressions had a closer fit to the data at the lower AA intakes (Figure VII.2) and therefore gave better estimates of CAAD.

A possible error in the estimates of CAAD is the assumption that there is no increase in the amount of AA excreted on diets containing greater levels of protein. There has been work that suggests a large amount of the increased AA excretion is of endogenous origin (Crompton

and Nesheim, 1969; Low, 1982). If there were an increase in the excretion of endogenous AA upon increased protein intake the actual CAAD would be greater than the present estimates. There is however no concise evidence on whether enzyme secretion into the gastrointestinal tract, which makes up a large part of endogenous secretion, has one consistent respones to AA or dietary intake (Snook, 1974; Bird and Moreau, 1978; Corring, 1981; Partridge et al., 1982). In the case of the birds fed dried egg albumen there was a 18-fold increase in the level of AA excreted from the ileum (Table VII.7) as the level of protein intake increased from 0 to 40%.

In an attempt to determine whether the AA excreted were derived from endogenous AA or were more closely related to the dietary AA, the AA levels in egg albumen and the AA levels in the ileal digesta excreted on the N-free diet were divided by the AA levels in the ileal digesta of birds fed the diet containing 40% egg albumen, to obtain two sets of ratios (Table VII.8). Coefficients of variation were than determined to see which set of ratios had the least variation. The results indicated that the pattern of AA excreted on the diet containing 40% egg albumen more closely resembled that of egg albumen than that of the endogenous AA excretion (Table VII.8). This does not establish that there was no increase in the amount of endogenous AA excretion on higher protein diets but does indicate that most of the increased AA excreted when higher levels of protein were fed was of dietary origin.

D. Summary

The results of the experiments indicated that when ileal and fecal sampling were used to estimate CAAD of soybean meal there was a decrease in AA digestibility as AA intake increased. In contrast when total fecal collections were used there was little influence of intake on CAAD. The ileal sample collections of egg albumen also had a significant decrease in CAAD as AA intake increased. Sampling error did not appear to be the cause of the curvilinear response, of decreased AA digestibility as AA intake increased, in the sample collections. The use of logarithmic regressions did not eliminate all of the errors encountered when using the quadratic regressions. A comparison of the AA levels in egg albumen and those found in the

excreta would suggest a major portion of the excreted AA was of feed origin. The problems encountered while interpreting the data demonstrate the lack of an accurate measurement of endogenous AA excretion is a basic error in all estimates of AA digestibility.

Table VII.1 Composition of experimental diets.

	Diet No.									
	Trial 1					Trial 2				
Ingredients %	1	2	3	4	5	6	7	8	9	10
Corn starch	87	66	45	24	3	86	76	66	56	46
Dried Egg Albumen[1]	-	-	-	-	-	-	10	20	30	40
Soybean meal	-	22	44	66	88	-	-	-	-	-
Cellulose[2]	4	3	2	1	-	5	5	5	5	5
Constant ingredients[3]	9	9	9	9	9	9	9	9	9	9

[1]Highland Produce Ltd., Two Hills, Alberta.

[2]Alphafloc; Brown Company, Berlin, New Hampshire.

[3]Supplied the following levels per kg of diets: corn oil 31g; ground limestone 8g; Biofos (18% Ca-21% P) 33g; iodized salt 6g; chromic oxide 1 g; vitamin-mineral premix 11g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D_3 600 ICU; vitamin E 10 IU; menadione 3mg; riboflavin 6mg; calcium pantothenate 10mg; niacin 15mg; folic acid 1mg; biotin .2mg; vitamin B_{12} 10mcg; choline chloride 1500mg; manganese 100mg; zinc 80mg.

TABLE VII.2. Quadratic, linear and logarithmic regressions between total amino acid excreted and total amino acid intake per 100 grams feed intake for ileal and fecal digesta sample collections and total fecal collections.

Analysis	b_0	b_1x	b_2x^2	F† (probability)	r^2
Sample Ileal					
Quadratic	.883±.257‡	.032± .039	.004 ± .001	15.7 (.0014)	.96
Linear	.381±.314	.182± .015			.91
Logarithmic	.028±.120	.056± .005			.93
Sample Fecal					
Quadratic	1.416 ± .320	.026 ± .049	.006 ± .001	19.3 (.0006)	.94
Linear	.722±.415	.181± .020			.85
Logaritthmic	.049±.113	.057± .005			.94
Total Fecal					
Quadratic	1.503 ± .210	.070 ± .032	.002 ± .001	3.6 (.077)	.89
Linear	1.306 ± .198	.129 ± .010			.88
Logarithmic	.602 ± .115	.031 ± .005			.81

†F-value and probability of quadratic regression coefficient (B_2) equaling zero.

‡Regression coefficients ± standard error.

Table VII.3 The amino acid digestibilities derived using the integrals and derivatives of the regressions of soybean meal at different levels of amino acid intakes.

		Digestibilites			
		Quadratic		Logarithmic	
Intake g/100g	Excretion g/100g	Derivative %	Area %	Derivative %	Area %
Sample Ileal					
0	.7	-	-	-	-
10.0	1.6	88.1	93.9	89.8	92.9
16.9	3.2	82.0	91.9	85.0	91.8
28.6	4.5	71.8	88.5	71.0	89.3
35.4	8.0	65.8	86.5	57.5	87.3
Sample Fecal					
0	1.4	-	-	-	-
10.0	1.8	90.5	98.6	89.3	92.6
16.9	3.0	82.1	95.6	84.1	91.4
28.6	5.2	68.0	91.0	68.9	89.3
35.4	8.3	59.7	88.3	54.0	86.6
Total Fecal					
0	1.8	-	-	-	-
10.0	2.3	89.6	91.8	92.3	93.1
16.9	2.9	87.2	91.1	90.5	92.0
28.6	4.1	83.3	89.7	86.4	89.8
35.4	5.8	81.0	89.0	83.2	88.3

Table VII.4 A comparison of dry matter digestibilities of diets containing different levels of soybean meal using different collections methods.

% Soybean Meal %	Sample Ileal %	Sample Fecal %	Total Fecal %
0	73.7 ± 3.2	76.4 ± 4.6	77.0 ± .6
22	74.9 ± .2*b*	76.1 ± .2*a*	75.9 ± 2.3*ab*
44	67.4 ± 1.5*b*	72.0 ± 1.1*a*	70.8 ± .9*a*
66	55.3 ± 1.7*b*	65.2 ± 0.4*a*	57.7 ± 3.9*b*
88	39.8 ± 4.0*b*	56.9 ± 1.0*a*	50.8 ± 8.0*a*

a,b Means of dietary treatments followed by different subscripts are signficantly different $P < .05$.

Table VII.5 Influence of collection method on the excretion of amino acids on a dry matter basis from diets containing different levels of soybean meal.

% Soybean Meal %	Sample Ileal %	Sample Fecal %	Total Fecal %
0	3.5 ± .7*b*	6.5 ± 2.8*a*	8.2 ± 1.3*a*
22	7.7 ± .9*b*	7.3 ± 1.3*b*	10.6 ± .9*a*
44	9.9 ± 1.1*b*	12.3 ± .8*b*	10.7 ± .9*ab*
66	12.4 ± .9	13.6 ± .9	11.2 ± 1.1
88	14.1 ± 1.5*ab*	17.5 ± 1.8*a*	12.5 ± .6*b*

a,b Means of dietary treatments followed by differents subscripts are signficantly different $P<.05$.

Table VII.6 Quadratic and logarithmic regressions between total amino acid excreted and total amino acid intake per 100 grams feed intake for ileal digesta sample collections of dried egg albumen.

Analysis	b_0	b_1x	b_2x^2	r^2
Quadratic	1.153 ± .666[1]	-.264 ± .104	.021 ± .003	.95
Logarithmic	-.513 ± .118	.0994 ± .0065		.95

[1]Regression coefficients ± standard error.

Table VII.7 The amino acid digestibilities derived using the integrals and derivatives of the regressions of dried egg albumen at different levels amino acid intakes.

		Digestibilites			
		Quadratic		Logarithmic	
Intake g/100g	Excretion g/100g	Derivative %	Area %	Derivative %	Area %
0	.7	-	-	-	-
7.6	1.4	110.1	115.6	87.3	92.2
15.1	2.0	94.1	104.9	73.3	89.5
22.7	5.2	77.8	94.0	43.1	85.3
30.2	13.2	61.7	83.3	-19.8	78.7

Table VII.8 Coefficient of variation of the ratios between dietary and endogenous amino acids and amino acid excretion on the diets containing 40% egg albumen.

Amino acid	Feed/Fecal	Endogenous/Fecal
Aspartic acid	1.98	.0042
Threonine	1.99	.0795
Serine	2.10	.0473
Glutamic acid	2.24	.0424
Proline	1.88	.0776
Glycine	1.97	.0654
Alanine	2.35	..0464
Valine	2.26	.0359
Methionine	2.63	.0181
Isoleucine	2.12	.0439
Leucine	2.56	.0487
Tyrosine	2.42	.0619
Phenylalanine	2.26	.0622
Lysine	2.60	.0387
Histidine	2.73	.0444
Arginine	2.25	.0490
Total	2.27	.0504
s	2.60	.0157
C.V.=(sx100)/mean	11.5	31.1

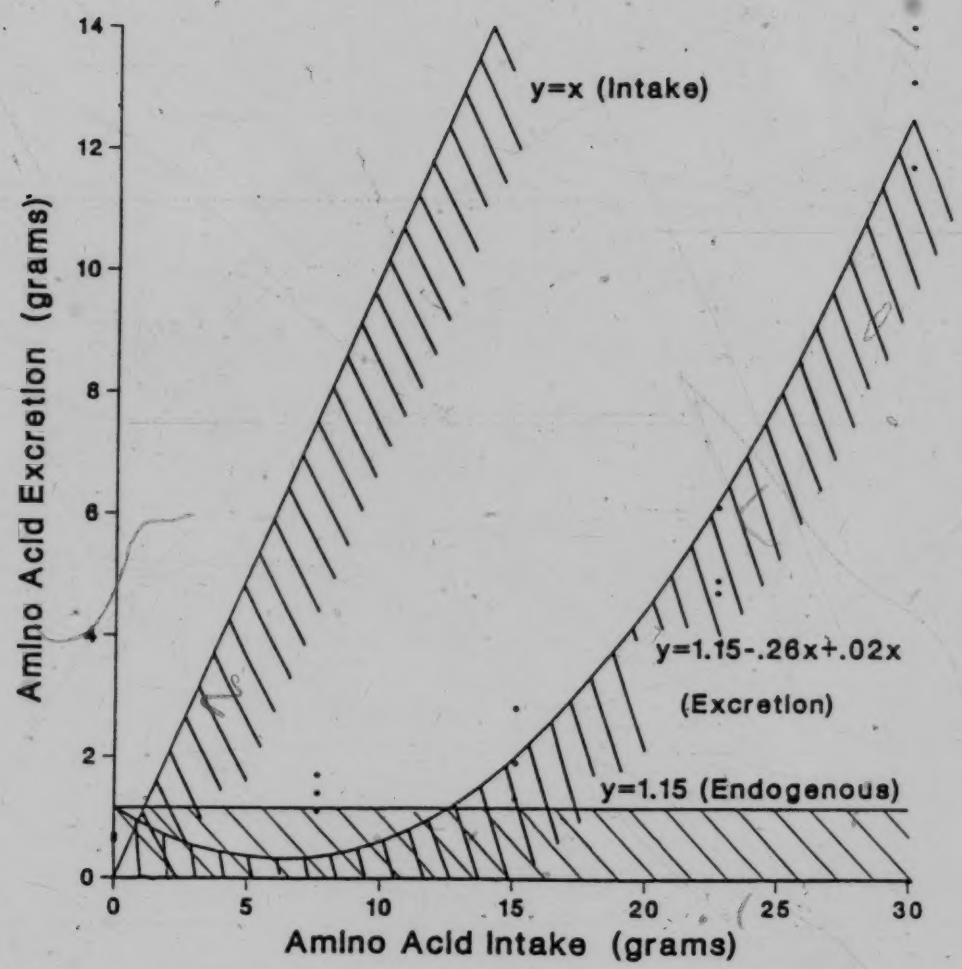

Figure VII.1 The quadratic regression of total amino acid excretion versus total amino acid intake of egg albumen. The areas underneath the regressions were used to calculate total amino acid digestibility. Digestibility = Intake - (Excretion-Endogenous).

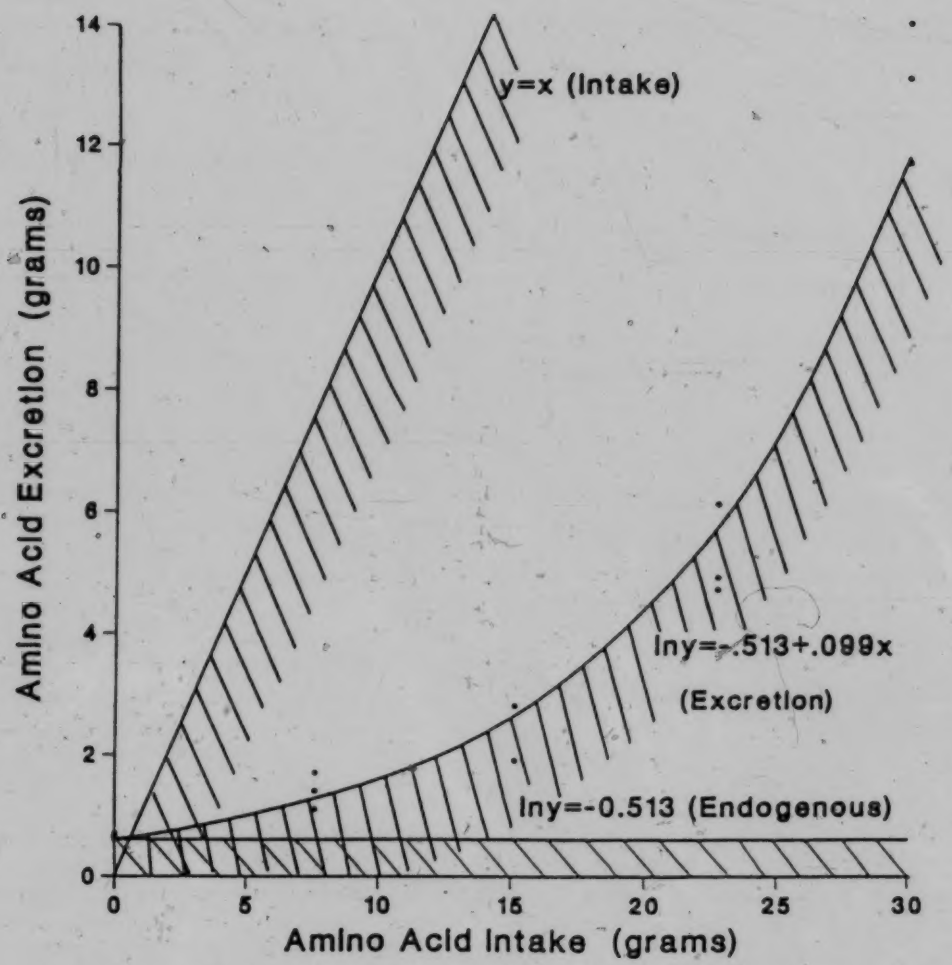

Figure VII.2 The logarithmic regression of total amino acid excretion versus total amino acid intake of egg albumen. The areas underneath the regressions were used to calculate total amino acid digestibility. Digestibility = Intake (Excretion-Endogenous).

VIII. Influence of Dietary Fat Supplementation of Laying Hen Diets on Protein Utilization.

A. Introduction

Numerous studies have shown the inclusion of fat in diets of laying hens has an "extra caloric" effect (Jensen et al., 1970; Sell et al., 1976; Mateos and Sell, 1980a). This "extra caloric" effect has been reported to be due to an increase in the available metabolizable energy from the non-fat portion of the diet perhaps resulting from a decrease in the rate of passage of the diet (Mateos and Sell, 1980b; Mateos and Sell, 1981). If fat has such an effect on the available metabolizable energy in a diet, it might be postulated that the addition of dietary fat may also enhance the available protein of the diet. Tao et al. (1971) in diets containing rapeseed meal showed an apparent increase in the availability of dietary protein when fat was added to the diet

The inclusion of fat in the diet has also been shown in some instances to influence egg weight. Most of the increase in egg weight upon the addition of fat to a diet has been attributed to an increase in dietary linoleic acid rather than an increase in energy content of the diet (Reid and Weber, 1975; Whitehead, 1981). Most of this increase in egg weight has been noted at total linoleic acid levels up to 1% of the diet The addition of animal tallow to laying diets has had variable results on egg weight (Jackson et al., 1969; Reid and Weber, 1975; Jones et al., 1976). The addition of tallow has not had as great an effect on egg weight as vegetable oil, but additions up to 3.5% have resulted in some egg weight increase over diets containing no tallow addition. At adequate protein intakes, the effect of energy "per se" has been reported to have little or no effect on egg weight or rate of egg production (Balnave, 1972; Jones et al., 1976; Carew et al., 1980; Reid and Maiorino, 1980). Numerous studies have shown, however that when protein intake is inadequate a decrease in egg weight and (or) rate of production may occur (Gabriel and Fetuga, 1976; Gleaves et al., 1977; Reid and Mairorino, 1980; Roland, 1980).

The present study was designed to investigate the effects of dietary fat levels on protein utilization in diets of laying hens formulated either to just meet recommended calorie-protein (C/P) ratios or to have slightly wider C/P ratios. It was postulated that the wider C/P ratio would result in a reduction of protein intake and therefore any improvement in protein availability resulting from the addition of fat should result in an increased egg weight and (or) rate of egg production.

B. Materials and Methods

Four hundred and eighty Shaver Starcross pullets were selected from pullets reared during the growing period in floor pens and given a commercial type grower diets. At 20 weeks of age the pullets were randomly divided into 32 groups of 15 birds each and the birds were housed in individual cages (30cm x 46cm). Two groups of birds were placed on each of the 16 experimental diets at 23 weeks of age for a 16-week experimental period. Feed and water were supplied ad libitum and the birds were given 14 hours of light per day.

Records were kept on daily egg production and mortality. Egg weights were taken once a week using the average egg weight of one day's production for each group of birds. Feed consumption was recorded for each four week period. Initial and final body weights were recorded.

The diets (Table VIII.1) were formulated to maintain comparable C/P ratios with increasing levels of added tallow. The diets were based on either wheat and canola meal or wheat and soybean meal. The ingredients used were analyzed for protein content and the metabolizable energy values used were those used at the University of Alberta (Robblee, unpublished). Within each ingredient base a series of diets with four levels of added tallow (0,3,6 and 9%) were formulated to contain C/P ratios of either 190:1 or 210:1 (kcal. M.E./%crude protein /kilogram of diet). The C/P ratios were kept constant by adjusting the levels of the wheat, soybean meal or canola meal, tallow and sucrose. Corn oil was added to all diets at a level of 1% to ensure that linoleic acid was not a factor influencing egg weight.

The data were analyzed by analysis of variance and means were compared using the Student-Newman-Keuls' test as outlined in Steel and Torrie (1980).

C. Results and Discussion

The effect of tallow levels and C/P ratios on caloric and protein intakes, egg weight, egg production and body weight are summarized in Table VIII.2. The levels of tallow used did not significantly affect caloric or protein intake, whereas differences in the C/P ratios had a significant effect. Birds fed the diets with the wider C/P ratio, (210:1), had greater caloric intakes, but even with the increased caloric intakes, protein intakes remained significantly lower than that of the birds fed diets with the narrower C/P ratio, (190:1). The increased caloric intake may have occurred because of sub-marginal protein intakes on the diets with the wider C/P ratio. Reid (1976) and Cherry et al.(1984) have shown that laying hens will increase their caloric intake in order to try to meet their protein requirement.

The diets with the narrower C/P ratios, (190:1), were based on recommended C/P ratios of 1:170-190 (Scott et al., 1976; NRC.,1977), and should have been adequate in protein, whereas the diets with the wider C/P ratios, (210:1), should have been sub-marginal in protein content. In this experiment, however, average caloric intakes of 337 and 342 kcal M.E./hen/day on the diets with the C/P ratios of 190:1 and 210:1 respectively, were higher than the expected caloric intake of approximately 325 kcal M.E./hen/day (Scott et al., 1976). As a consequence the average protein intakes of 17.7 and 16.3 grams/hen/day for the 190:1 and the 210:1 diets were also higher then expected. Despite this there was a significant decrease in egg weight of the hens fed diets with the 210:1 ratios as compared to those fed the diets with 190:1 ratios. No significant differences in rate of egg production was observed, between the groups fed the diets with the narrower (190:1) and wider (210:1) C/P ratios.

Egg weight and rate of production were not influenced by the dietary tallow levels used (Table VIII.2). It therefore appears that the addition of tallow had no effect on protein availability. If the addition of tallow increased protein availability, some increase in egg weight

or production rate might have been expected on the diets with the wider C/P ratio but no such increase was noted.

The only significant effect of tallow addition was on weight gain, which increased with each level of tallow addition up to 6% of the diet Cunningham and Morrison, (1977) have shown that most of the body weight gain that occurs during the laying year is due to excess energy which is deposited as fat. In the present experiment the body weight gain was similar with both C/P ratios, suggesting that weight gain was due to the "extra caloric" effect of fat rather than an increase in protein availability.

Soybean meal and canola meal were used as protein sources to see if the difference protein availabilities as reported by Muztar et al. (1980) would be affected by dietary tallow level and therefore be reflected in egg weight or egg production differences. The only effect noted with higher levels of tallow was a slight increase in egg weight on the canola meal diets (Table VIII.3). There was however no further increase in egg weight with dietary levels of tallow above 3%. It is therefore unlikely that the increase in egg weight that occurred was a result of increased protein availability.

The hens fed diets containing canola meal had significantly lower egg weights and lower rate of egg production than those fed diets containing soybean meal. They also had significantly lower average caloric intakes, 332 versus 346 kcal M.E./hen/day than hens fed the soybean meal diets. The decreased caloric intakes of birds on the canola meal diets were consistent across all tallow levels and C/P ratios. Previous work has shown that the caloric intakes on canola meal diets were lower than on soybean meal diets (March, 1981).

The decreased feed intake that occurred on the canola meal diets resulted in decreased protein intake as compared to the hens fed the diets containing soybean meal. When protein efficiency was evaluated by dividing total egg mass (egg weight x egg production) by grams of protein intake there was, however, no significant difference between the canola meal and the soybean meal diets. An interesting speculation is that if the canola meal diets had equal protein intakes would there be any decrease in egg weight and production? This question can be

answered in part by the results noted with the different C/P ratios. An increase in protein intake of 1.4g/hen/day on the diets with a C/P ratio of 190:1 resulted in only a 0.7g increase in egg weight and a non-significant increase of 0.7% in egg production as compared to the performance of hens fed the diets with a C/P ratio of 210:1. Hens fed the soybean meal diets had an average increased protein intake of only 0.7g/hen/day resulting in a 1.6g increase in egg weight and a 1.2% increase in egg production. This suggests the increased protein intake of the soybean meal diets was not the only cause for the increase in egg weight and production noted. Protein digestibility, quality or other factors, such as goitrogens in the canola meal, may have also been factors influencing egg weight and production.

D. Summary

The addition of increasing levels of tallow (0, 3, 6, and 9%) to laying diets with different C/P ratios and containing either soybean meal or canola meal as protein sources had no significant effects on rate of egg production or egg weight. There was, however, a significant increase in body weight gain with higher levels of tallow in the diet which was attributed to increased energy utilization rather than improved protein utilization. The hens fed the wider (210:1) C/P ratio had lower caloric and protein intakes which resulted in a decrease in egg weight. The hens fed the canola meal diets had lower caloric and protein intakes resulting in a decrease in egg weight and egg production.

TABLE VIII.1. Composition of experimental diets.

Diet	1	2	3	4	5	6	7	8
Wheat	54.2	55.9	57.6	59.3	49.5	51.2	52.7	54.4
Cellulose[1]	2.0	2.0	2.0	2.0	2.2	2.2	2.2	2.2
Sucrose	15.4	10.3	5.1	-	21.2	16.1	11.1	6.0
Soybean meal	14.5	14.9	15.4	15.8	13.2	13.6	14.1	14.5
Premix[2]	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9
Stabilized tallow[3]	-	3.0	6.0	9.0	-	3.0	6.0	9.0
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Calculated Analysis								
Met. Energy kcal/kg	2767	2853	2940	3027	2810	2897	2985	3073
Protein %	14.56	15.02	15.48	15.93	13.36	13.79	14.20	14.63
Calorie-Protein Ratio	190	190	190	190	210	210	210	210

Diet	9	10	11	12	13	14	15	16
Wheat	47.3	48.8	50.4	51.9	43.5	44.9	46.3	47.7
Cellulose	-	-	-	-	.5	.5	.5	.5
Sucrose	18.5	13.4	8.1	3.0	23.5	18.5	13.5	8.5
Canola meal	20.3	20.9	21.6	22.2	18.6	19.2	19.8	20.4
Premix	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9
Tallow	-	3.0	6.0	9.0	-	3.0	6.0	9.0
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Calculated Analysis								
Met. Energy kcal/kg	2692	2777	2860	2944	2739	2824	2910	2993
Protein %	14.14	14.59	15.03	15.48	13.03	3.45	13.86	14.27
Calorie-Protein Ratio	190	190	190	190	210	210	210	210

[1]Alfafloc; Brown Company, Berlin, New Hampshire.

[2]Supplied the following levels per kg of diets:corn oil 10g; ground limestone 40g; oyster shell 40g; Biofos (18% Ca-21% P) 15g; iodized salt 3g; alfalfa 10g; D-L methionine 0.5g; lysine-HCl 0.5g; vitamin-mineral premix 20g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D_3 600 ICU; vitamin E 5 IU; riboflavin 4mg; calcium pantothenate 6mg; niacin 15mg; vitamin B_{12} 10 mcg; choline chloride 100mg; biotin 100mg; manganese 100mg; zinc 75mg.

[3]Animal tallow.

TABLE VIII.2 Effect of tallow levels and calorie-protein ratios on hen performance.

% Tallow	190:1					210:1				
	0	3	6	9	Mean	0	3	6	9	Mean[1]
Feed intake g/hen/day	123.8*a*	117.0*b*	114.0*c*	111.0*d*	116.4	123.3*a*	118.8*b*	112.6*c*	110.4*d*	116.3
Energy intake kcal/hen/day	338.8	332.7	336.2	339.3	336.8	342.7	342.9	337.3	343.9	341.5*
Protein intake g/hen/day	17.9	17.5	17.6	17.7	17.7	16.3	16.2	16.0	16.2	16.3**
Egg weight g/egg	55.4	56.3	56.9	56.8	56.4	55.7	55.5	56.2	55.4	55.7*
Egg production (henday) %	91.1	90.9	92.4	91.8	91.6	91.9	93.5	87.0	91.1	90.9
Body weight gain, g	205*a*	246*b*	290*c*	277*c*	255	188*a*	231*b*	252*c*	257*c*	232
Total egg Mass[2]/ Daily Protein Intake	2.79	2.91	2.98	2.95	2.91	3.09	3.16	3.04	3.12	3.10**

a,b,c,d Means with different superscripts within a C/P ratio differ significantly ($P<0.05$).

[1]Calorie-Protein ratio means with * are different at ($P<0.05$) and ** ($P<0.01$).

[2]Total egg mass = egg weight x egg production.

TABLE VIII.3. Effect of tallow levels and soybean meal and canola meal on hen performance.

% Tallow	Soybean meal					Canola meal				
	0	3	6	9	Mean	0	3	6	9	Mean[1]
Feed intake g/hen/day	125.3*a*	118.2*b*	113.5*c*	112.1*d*	117.8	121.8*a*	117.5*b*	113.0*c*	109.3*d*	115.4*
Energy intake kcal/hen/day	349.6	342.9	341.8	349.9	346.1	331.9	332.7	331.7	332.4	332.2**
Protein intake g/hen/day	17.8*a*	17.3*b*	17.1*b*	17.3*b*	17.4	16.8	16.7	16.6	16.5	16.7**
Egg weight g/egg	56.9	56.6	57.2	56.6	56.8	54.2*a*	55.2*b*	55.9*b*	55.6*b*	55.2**
Egg production (henday) %	91.6	93.3	90.8	92.8	92.1	91.4	91.1	88.6	90.1	90.9*
Body weight gain, g	240*a*	281*b*	322*c*	357*d*	300	154*a*	196*b*	220*d*	170*c*	187**
Total egg Mass[2]/ Daily Protein Intake	2.93	3.06	3.04	3.02	3.01	2.95	3.01	2.98	3.04	3.00

a,b,c,d Means with different superscripts within a protein supplement differ significantly ($P<0.05$).

[1]Protein supplement means with * are different at ($P<0.05$) and ** ($P<0.01$).

[2]Total egg mass = egg weight x egg production.

IX. Effect of Feeding Diets Containing Soybean and Canola Meal at Various Calorie-Protein Ratios on the Performance of Laying Hens.

A. Introduction

In the study on the use of fat in diets of laying hens (Chapter 8) the performance of the birds was reduced by using diets containing canola meal. How much of the decline in hen performance could be attributed to the lower amino acid digestibility of canola meal could not be determined. It was observed, however, that the use of wider calorie-protein (C/P) ratios in rations containing either soybean meal or canola meal resulted in decreased amino acid (AA) intakes. To further evaluate the effect of substituting canola meal for soybean meal as an AA source in laying diets an experiment was conducted in which canola and soybean meal were included in diets with different C/P ratios. In the diets equivalent amounts of protein were contributed by wheat and either canola meal or soybean meal within each C/P ratio. A second experiment was conducted to determine the corrected amino acid digestibility (CAAD) of canola meal and soybean meal for laying hens. Using the information on digestible AA the performance of the hens fed diets containing canola meal or soybean meal was compared.

B. Materials and Methods

In the first experiment 480 Shaver Starcross pullets were housed in individual cages (30cm x 46cm) at 20 weeks of age and fed a laying ration. At 30 weeks of age the pullets were randomly divided into 16 groups of 30 birds each. Two groups of birds were placed on each of the 8 experimental diets. The experiment lasted for 12 weeks.

While on experiment the birds were given feed and water ad libitum and provided with fourteen hours of light daily. Records were kept on daily egg production and mortality. Egg weights were taken once a week using the average egg weight of one day's production for each group of birds. Feed consumption was recorded for each four week period. Initial and final body weights were taken.

The diets fed (Table IX.1) were isocaloric and were formulated using either wheat and canola meal, or wheat and soybean meal as the protein source. Within each protein source diets were designed to provide C/P ratios of 190:1, 200:1, 210:1 or 220:1 (kcal M.E./% crude protein /kilogram of diet). The AA profiles for each protein source were kept constant by having the same amount of protein provided by wheat and the same amount provided by either canola meal or soybean meal within each C/P ratio. It was therefore postulated that differences in hen performance within each C/P ratio should be due to either the canola meal or the soybean meal component of the diets fed. One percent corn oil was added to all diets as a source of linoleic acid.

In the second trial 36 hens of 43 weeks of age and comparable weight were randomly divided into 12 groups of 3 hens each and placed in cages (76cm x 76cm) equipped to collect fecal material. Three cages of three birds were placed on each of the 8 diets. The composition of the experimental diets is given in Table IX.2. The four diets contained 11.6% fixed ingredients with the remainder consisting of autoclaved corn starch, wheat, soybean meal or canola meal. The N-free diet containing 86% autoclaved corn starch was required to estimate endogenous AA excretion. Chromic oxide was added as an inert marker. The hens were starved for 12 hr before being placed on the experimental diets for 12 to 13 hr. For the last two hours of the feeding period trays were placed under the birds to collect fecal material. After the 12 hr feeding period the birds were sacrificed by cervical dislocation and the contents from the terminal 18cm of the ileum was removed during the following hour for analysis following the same procedure used for broiler chicks in Chapter 4.

Fecal and ileal samples from each group were freeze-dried and weighed and ground in a Universal micro mill for 5 minutes. Dry matter, chromic oxide and AA content were determined on both feed and digesta samples. Chromic oxide was determined by the method of Fenton and Fenton (1979). The amino acid analyses of the feed and intestinal samples were done using the method outlined by Blackburn (1968). The samples were hydrolyzed by refluxing with 6N HCl for 24 hr and their amino acid content was determined using a

Beckman 121 MB amino acid analyzer.

To correct for endogenous amino acids the following equation was used to determine corrected amino acid digestibilities.

$$CAAD = \frac{\text{feed a.a.} - ((A/B \times \text{fecal a.a.}) - (C/D \times \text{N-free fecal a.a.})) \times 100}{\text{feed a.a.}}$$

A = feed Cr_2O_3

B = fecal Cr_2O_3

C = N-free Cr_2O_3

D = N-free fecal Cr_2O_3

The data were subjected to analysis of variance and means were compared using the Student-Newman-Keuls' text as outlined in Steel and Torrie (1980).

C. Results and Discussion

The influence of C/P ratio and protein source on hen performance is shown in Table IX.3. Although the diets were isocaloric there was an increase in daily feed intake on both the canola meal and the soybean meal diets with the wider C/P ratios (210:1 and 220:1) than with the narrower C/P ratios (190:1 and 200:1). As the C/P ratios widened there was a significant decrease in daily protein intake, except for the 200:1 and 210:1 C/P ratios in which the increase in feed intake of the 210:1 diets offset the effect of the lower protein content in the diets. Protein source did not influence daily protein intake. At each C/P ratio, intakes were similar on diets containing canola meal and soybean meal.

Hens fed the diets containing canola meal showed a decrease in egg weight between the 190:1 and 200:1 C/P ratio diets and a decrease in egg production when the C/P ratio was widened from 210:1 to 220:1. This agrees with other observations (Balloun and Speers, 1969) that when dietary protein becomes limiting a decrease in egg weight occurs before a decrease in egg production. The canola meal diets therefore appeared to supply adequate intakes of digestible AA on the 190:1 diet and then as protein intake decreased one or more of the AA

became limiting and hen productive performance declined.

The hens fed the soybean meal diets did not show any decrease in egg weight as the C/P ratios were widened. There was, however, a decrease in egg production by birds fed the diet with a C/P ratio of 200:1 as compared to those with a C/P ratio of 190:1. Since there was no further decrease in egg production or egg weight, as the C/P ratio was widened the higher egg production on the 190:1 C/P diet cannot be explained.

In the second experiment the ileal and fecal CAAD values for canola meal, soybean meal and wheat are presented (Table IX.4). In the three feedstuffs the total ileal CAAD values were lower then the fecal values. The ileal and fecal CAAD values for canola meal were 74.2 and 79.2%, for soybean meal 91.6 and 92.9%, and for wheat 84.7 and 96.3%, respectively. Raharjo and Farrell (1981) had found similar differences between derived ileal and fecal apparent AA digestibilities.

The fecal CAAD values for canola meal ranged from 69.8% for aspartic acid to 87.3% for methionine. This range is in reasonable agreement with the range of fecal CAAD found by Tao et al. (1971) of 59.8 to 81.9% and also with one set of values reported by Muztar et al. (1980) of 67 to 83%. In other reports, fecal CAAD values for canola meal were much higher ranging from 85 to 95%, (Nwokola et al., 1976; Muztar et al., 1980). There was , however, no consistency in the individual CAAD values which varied between the reported values.

The range of digestibility of individual AA for soybean meal were similar to other reported values (Achinewhu and Hewitt, 1979; Sibbald, 1979b; Muztar et al., 1980), with average CAAD values of 92-93%, and little or no differences between individual CAAD. The differences between the ileal and fecal CAAD values were also very small in comparison to canola meal.

The individual CAAD values for wheat showed a large difference between the ileal and fecal values. Because of the range in CAAD reported in the literature some of the reported CAAD values agree more with ileal CAAD values and others agree more with fecal CAAD values (Sibbald, 1979a; McNab and Shannon, 1974: Achinewhu and Hewitt, 1979).

Based on NRC (1977) requirement levels the most limiting AA in the canola meal diets were leucine, lysine and arginine, while in soybean meal the limiting AA were leucine and methionine. In Table IX.5 the calculated digestible AA intakes based on the ileal CAAD of the diets are presented. According to the calculated values of digestible AA intake, the canola meal diets with C/P ratios of 200:1 and 210:1 should have performed as well as the soybean meal diet with the C/P ratio of 220:1. Thus the total digestible AA may not have been the only factor causing the decrease in hen performance on the canola meal diets. It is possible that one or a combination of the limiting AA in the canola meal diets may have caused the decrease in hen performance.

The intakes of digestible leucine, lysine and arginine on the canola meal diets were below those of the lowest digestible AA intake on the soybean meal diets. The possibility exists that other factors may have caused the the decreased performance on the canola meal diets in comparison to the soybean meal diets. Although many of the adverse effects of rapeseed meal in poultry diets have been eliminated or reduced by the new canola varieties, higher levels of phytic acid, erucic acid, glucosinolates and fiber may have been responsible for the decrease in laying hen performance when canola meal was added to the diets. Nevertheless the differences in intake of digestible AA may account for most of differences found in the performance of laying hens when fed diets with differing C/P ratios and containing canola or soybean meal as protein supplements. Consequently calculation of the intake of digestible AA may prove to be very useful in formulating efficient laying rations.

D. Summary

The diets containing canola meal had a decrease in egg weight and egg production as the C/P ratio widened. The diets containing soybean meal did not have an apparent decrease in egg weight or egg production. The ileal CAAD of canola meal, soybean meal and wheat were found to be lower than the fecal CAAD. The total CAAD for the ileal and fecal digesta was 74.7 and 79.2% for canola meal, 91.6 and 92.9% for soybean meal, and 84.7 and 96.3% for wheat

respectively. The difference in intake of the total digestible AA could account for most of the differences in hen performance between diets containing canola meal and soybean meal, and in those with the different C/P ratios.

TABLE IX.1. Experimental diets.

Diet	1	2	3	4	5	6	7	8
Soybean meal	15.3	-	14.5	-	13.8	-	13.1	-
Canola meal	-	18.6	-	17.6	-	16.8	-	16.0
Wheat	61.1	61.4	58.0	58.3	55.2	55.4	52.6	52.8
Cellulose[1]	1.5	-	1.5	-	1.5	-	1.5	-
Sucrose	3.7	0.4	8.6	5.8	13.0	10.5	17.2	14.9
Biofos[2]	2.2	1.7	2.2	1.7	2.2	1.7	2.2	1.7
Premix[3]	13.9	13.9	13.9	13.9	13.9	13.9	13.9	13.9
Stabilized tallow[4]	4.6	6.3	3.6	5.0	2.7	4.0	1.8	3.0
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
Calculated Analysis								
Met. Energy kcal/kg	2851	2850	2851	2850	2851	2850	2850	2850
Protein %	15.00	15.00	14.25	14.25	13.57	13.57	12.94	12.94
Calorie-Protein Ratio	190	190	200	200	210	210	220	220

[1]Alfafloc; Brown Company, Berlin, New Hampshire.

[2]Biofos (18% Ca-21% P)

[3]Supplied the following levels per kg of diets:corn oil 10g; ground limestone 82g; iodized salt 3.5g; D-L methionine 0.5g; vitamin-mineral premix 20g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D, 600 ICU; vitamin E 5 IU; riboflavin 4mg; calcium pantothenate 6mg; niacin 15mg; vitamin B_{12} 10 mcg; choline chloride 100mg; biotin 100mg; manganese 100mg; zinc 75mg.

[4]Animal tallow.

Table IX.2 Composition of experimental diets for digestibility trials.

Ingredients %	Diet No. 1	2	3	4
Corn starch	86	-	-	20
Wheat	-	86	-	-
Canola meal	-	-	86	-
Soybean meal	-	-	-	66
Cellulose[1]	5	5	5	5
Constant ingredients[2]	9	9	9	9

[1]Alphafloc; Brown Company, Berlin, New Hampshire.

[2]Supplied the following levels per kg of diets: corn oil 31g; ground limestone 8g; Biofos (18% Ca-21% P) 33g; iodized salt 6g; chromic oxide 1 g; vitamin-mineral premix 11g. The vitamin-mineral premix supplied the following levels per kg of diet: vitamin A 4000 IU; vitamin D_3 600 ICU; vitamin E 10 IU; menadione 3mg; riboflavin 6mg; calcium pantothenate 10mg; niacin 15mg; folic acid 1mg; biotin .2mg; vitamin B_{12} 10mcg; choline chloride 1500mg; manganese 100mg; zinc 80mg.

TABLE IX.3. Effect of calorie-protein ratio and soybean meal and canola meal on hen performance.

Calorie-Protein Ratio	Soybean meal					Canola meal				
	190:1	200:1	210:1	220:1	Mean	190:1	200:1	210:1	220:1	Mean[1]
Feed intake g/hen/day	113.2*a*	112.8*a*	116.9*b*	115.9*b*	114.6	110.0*a*	109.5*a*	115.9*b*	115.7*b*	112.8
Energy intake kcal/hen/day	322.6	321.7	333.2	329.5	326.8	313.4*a*	312.1*a*	330.3*b*	329.7*b*	321.4
Protein intake g/hen/day	16.9*a*	16.0*b*	15.8*b*	14.4*c*	15.7	16.5*a*	15.7*b*	15.6*b*	14.8*c*	15.6
Egg weight g/egg	58.5	59.2	59.1	58.1	58.7	58.8*a*	57.3*b*	57.5*b*	57.0*b*	57.6**
Egg production (henday) %	89.3*a*	85.3*b*	85.9*b*	86.3*b*	86.7	85.0*a*	85.4*a*	85.9*a*	81.1*b*	84.3**
Body weight gain, g	553	522	532	496	526	492	487	467	427	468*
Total egg Mass[2]/ Daily Protein Intake	3.16*a*	3.16*a*	3.21*a*	3.49*b*	3.25	2.86*a*	3.11*b*	3.17*b*	3.11*b*	3.06**

a,b,c,d Means with different superscripts within a protein supplememt differ significantly ($P<0.05$).

[1]Protein supplements means with * are different at ($P<0.05$) and ** ($P<0.01$).

[2]Total egg mass = egg weight x egg production.

Table IX.4 Ileal and fecal amino acid digestibilities of the dietary feedstuffs.

Amino acid	Canola meal		Soybean meal		Wheat	
	Ileal	Fecal	Ileal	Fecal	Ileal	Fecal
Aspartic acid	68.2 ± .3[1]	69.8 ± 3.2	90.7± .2	89.2 ± 1.0	76.6 ± .5	91.8 ± .8
Threonine	71.6 ± .8	77.6 ± 2.0	89.0± .6	93.2 ± .6	93.9 ± 2.0	98.5 ± .5
Serine	73.0 ± .5	76.4 ± 1.4	93.0± .9	92.7 ± .6	89.4 ± 1.7	94.1 ± 1.8
Glutamic acid	81.4 ± 1.2	84.5 ± .9	94.1± .2	92.6 ± .9	91.0 ± 3.4	97.9 ± .1
Proline	73.6 ± 8.6	76.9 ± 1.2	92.7± .8	92.9 ± .8	91.4 ± 1.1	96.4 ± 1.1
Glycine	67.9 ± 2.7	-	89.0± .9	-	78.7 ± .4	-
Alanine	80.9 ± 2.0	84.7 ± 1.1	93.9± .3	94.1 ± .3	79.7 ± 2.7	92.7 ± 1.0
Valine	71.8 ± .2	71.8 ± 1.2	91.5± .5	89.4 ± 1.0	80.5 ± 2.6	95.8 ± 1.2
Methionine	82.6 ± .9	87.3 ± 1.2	88.6± .7	89.3 ± 1.2	74.4 ± .8	94.4 ± .6
Isoleucine	75.3 ± 1.3	76.6 ± 1.7	93.1± .5	91.7 ± .7	82.3 ± 3.5	96.0 ± .9
Leucine	79.0 ± 1.0	80.8 ± 1.6	93.3± .3	92.6 ± .3	84.2 ± 3.5	97.0 ± .4
Tyrosine	76.2 ± 4.1	78.5 ± 2.0	93.9± .4	93.6 ± 1.0	71.9 ± 2.0	96.9 ± .7
Phenylalanine	84.4 ± .7	80.7 ± 2.2	94.3± .3	94.1 ± .6	78.4 ±2.7	99.0 ± .9
Lysine	66.8 ± .8	78.8 ± 2.3	86.9± .4	94.2 ± .5	73.7 ± 4.1	91.5 ± 1.2
Arginine	79.8 ± .5	85.7 ± 2.0	86.5± 1.8	93.9 ± .8	89.5 ±4.1	93.3 ± .8
Total	74.7 ± .2	79.2 ± 1.6	91.6± .3	92.9 ± .7	84.7 ±2.6	96.3 ± .6

[1]Digestibility ± standard error.

Table IX.5 Grams of ileal digestible amino acid intake per day of selected amino acids and total amino acid based on ileal digestibilities.

	Arginine	Methionine	Leucine	Lysine	Total[1]
Soybean meal					
190:1	.95	.23	1.11	.65	13.56
200:1	.90	.22	1.05	.61	12.82
210:1	.89	.22	1.03	.60	12.64
220:1	.83	.21	.97	.57	11.89
Canola meal					
190:1	.84	.28	1.02	.55	12.62
200:1	.79	.27	.96	.52	11.91
210:1	.79	.27	.97	.52	12.01
220:1	.76	.26	.92	.50	11.42
NRC[2]	.88	.27	1.32	.60	16.00

[1]Total measured amino acids, does not included cysteine and histidine.

[2]NRC requirements are based on grams of amino acid intake, not on digestible amino acid intake.

X. General Discussion

The use of ileal digesta in the determination of corrected amino acid digestibilities (CAAD) of feedstuffs in this study established that differences can exists between ileal and fecal estimates. Depending on the protein source, the CAAD derived using ileal digesta were the same or lower than the fecal estimates. Soybean meal which has been established as a highly digestible protein source, showed little difference in CAAD values between ileal and fecal estimates. In contrast, canola meal, a less digestible protein had much lower CAAD values when derived from ileal digesta than those estimated using fecal collections. Although not directly comparable similar differences were found between the ileal and fecal CAAD of the cereals used. Wheat, a more digestible protein source, had little difference in CAAD values between ileal and fecal estimates, whereas oats had much larger differences between ileal and fecal CAAD estimates. Raharjo and Farrell, (1981) found similar differences between ileal and fecal amino acid digestibilities in plant protein of varying protein digestibilities.

Based on the level of amino acids (AA) found in the ileal digesta and in the fecal material, there appeared to be a net loss of AA in the hindgut when poultry were fed diets containing canola meal. In pigs it has been established that AA can be deaminated by the microflora and therefore cause a net loss of AA in the hindgut (Zebrowska, 1973; Henry, 1983). It has also been established in pigs that AA entering the hindgut cannot be absorbed into the blood stream and therefore cannot serve as a source of AA to the pig (Zebrowska, 1973; Sauer, 1976; Deguchi et al., 1978; Wunsche et al., 1982). In poultry, microflora have been shown to deaminate AA in the hindgut, but to a lesser extent (Salter and Coates, 1971; Parsons et al., 1982). Poultry have also been shown to have no absorption of AA from the hindgut (Salter and Coates, 1971; Furuse and Yorkota, 1984). The greater loss of AA on the canola meal diets therefore might be related to the larger amount of undigested AA entering the hindgut on the diets containing canola meal which would then be available for deamination. The greater the loss of AA in the hindgut through microbial deamination, the greater the difference will be between the ileal and fecal CAAD. Because of the microbial alteration of the

AA entering the hindgut the ileal AA digestibilities will provide a more accurate estimation of the availability of the AA to poultry.

Of particular interest when looking at the differences between the ileal and fecal excretion of AA was the differences in the levels of AA excreted on the N-free diets. The fecal excretion of AA per 100 grams feed intake was approximately twice the level found in the ileal digesta. Thus there would appear to be a large contribution of AA from the hindgut. Cecetomized birds fed a N-free diet have been shown to have greater losses of AA than intact birds (Darcy and Rerat, 1983). The additional AA excreted in the feces of intact birds, compared to the AA excreted from the ileum, would therefore not appear to have been from the ceca, which is the major site of microflora in the poultry hindgut (Fuller, 1984). The only other likely major source of AA is from the urine (Sykes, 1971). The effect of these additional excretions of AA from the hindgut of poultry could result in the fecal estimates of CAAD being higher than the ileal estimates. Even though the fecal excretion of AA on the N-free was greater than the levels in the ileal digesta this difference decreased as protein intake increased. As a consequence the correction for endogenous AA excretion was greater and therefore estimates of CAAD were higher with the fecal material.

The curvilinear relationship found between AA digested and AA intake differed from the linear relationship reported by Carlson and Bayley (1971), Sibbald (1979a), and Taverner et al. (1981). As the concentration of AA in the diets increased the proportion of AA found in the digesta increased at a greater rate. This then led to the curvilinear relationship between AA digested and AA intake. The regressions of AA digested on AA intake were significantly curvilinear only at intakes greater than 15% to 20% crude protein. Because of the curvilinear response in CAAD at the higher protein intakes, the use of linear regressions to estimate CAAD would lead to variation in the estimates depending on the levels of AA used in the experimental diets. For this reason curvilinear equations were fitted to the data points to try and obtain better estimates of CAAD. There were, however, also errors in fitting the curvilinear equations. The lack of sufficient data points led to problems in determining which

curve was the most representative of the true digestibility curve. In particular a lack of knowledge of the actual endogenous AA excretions resulted in errors in fitting digestibility curves. The result is that the total or average CAAD may be slightly higher or lower than the true CAAD, but the variation of the individual AA from these totals are still of value when comparing CAAD within a protein source. The importance of being able to establish whether a particular AA continually has higher or lower digestibilities than average CAAD should not be overlooked. In diet formulation the limiting essential AA are of most importance. As a consequence if an estimate of their digestibility can be established through determination of the crude protein digestibility then the need for routinely determining each of the individual CAAD can be eliminated.

Based on the experiments in this study it did not appear that the sampling of the ileal and fecal material was the cause of the curvilinear response in CAAD as AA intakes increased. The marker used (Cr_2O_3) seemed to provide a reasonable estimate of dry matter digestibility and factors such as time of sampling or length of starvation did not alter the concentration of AA found in the digesta. A difference in AA concentration in the digesta did exist, however, depending on whether sample ileal and fecal collections were used or total fecal collections were used. When total collections were employed there was a linear relationship between AA digested and AA intake and therefore AA intake did not affect the estimates of CAAD. There was no evidence however, to support the contention that one collection method gave more accurate estimates of CAAD than the other.

Although it did not appear that the curvilinear response in CAAD was due to sampling there are factors that might lead to variability. Differences in dietary intakes may affect the amount of excreted material and this in turn may influence the estimates of digestibility. The use of graded levels of test material in the experimental diets may have some effect upon the AA digestibility values obtained. In the case of total fecal collections the length of time that the fecal material remains on the collection trays before being frozen may affect CAAD estimates. Microbial breakdown of AA during this time would result in increased estimates of CAAD.

The major problem in all methods used to determine CAAD in poultry is accurately measuring the level of endogenous excretion of AA. The correction for endogenous AA is an estimate at best. Because of the inaccuracies surrounding the estimation of endogenous secretion of AA, some have suggested (Sauer, 1976; Darcy and Rerat, 1983) that a correction for endogenous AA may not be needed. This, however, results in large differences in estimates of AA digestibility especially for the highly digestible protein sources. In addition if values are not corrected for endogenous AA excretion the intake of AA becomes very important, when comparing different reported values. The use of a standard method for correcting for endogenous AA excretion allows for comparison between different reported values but does not necessarily give true digestibility values. The use of graded AA intakes in our experiments did not solve the problem of accurately measuring endogenous AA excretions, but did confirm that no matter how CAAD was measured, estimates of endogenous AA are very important. Without accurate measurements of endogenous AA excretions all reported CAAD may be higher or lower than true AA digestibility and therefore caution should be used when trying to compare reported values within or among protein sources.

A possible method for more accurately determining endogenous AA excretion may involved the use AA labeled with radioactive isotopes. Work conducted with ^{15}N labled AA (Krawielitzki, et al., 1977; Gebhardt et al., 1982) have shown that the digestibility of AA measured by ^{15}N labeled AA is higher than apparent AA digestibility. At the present time the cost of using ^{15}N labeled AA has prevented its widespread use in AA digestibility studies.

The supplementation of laying diets with tallow improved carbohydrate utilization but did not increase the protein utilization of the diets. This suggests that improved digestion of dietary carbohydrate source does not necessarily imply better digestion of dietary protein. The observation that lowering the percentage protein in the diets of laying hens caused them to increase their caloric intake presumably in an effort to meet their daily protein requirement agrees with other work that has been reported (Reid, 1976; Cherry et al., 1984). Eventually, however, a continued decline in dietary protein resulted in a decline in productive performance.

The decrease in productivity by the hens fed canola meal as a protein supplement as compared to those fed a diet containing soybean meal was mostly due to the lower digestibility of the protein in canola meal.

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XII. Appendix Tables

Table XII.1 Amino acid composition of the protein sources used in the experimental diets.

Amino acid	Fecal[1]			Ileal[2]			Soybean Meal	Canola Meal	Egg Albumen
	Wheat	Oats	Barley	Wheat	Oats	Barley			
Aspartic acid	.85	.90	.74	.78	.62	.78	5.10	2.98	8.22
Threonine	.43	.37	.42	.42	.37	.33	1.76	1.67	3.56
Serine	.80	.54	.52	.70	.44	.47	2.36	1.72	5.44
Glutamic acid	5.01	2.25	2.84	4.31	2.42	1.79	7.73	6.15	10.32
Proline	1.53	.69	1.22	1.16	.85	.42	2.00	2.51	2.91
Glysine	.67	.54	.48	.61	.40	.47	1.79	1.87	2.75
Alanine	.54	.52	.49	.49	.39	.43	2.30	2.00	4.62
Valine	.58	.57	.59	.59	.47	.45	1.65	1.82	4.92
Methionine	.19	.17	.17	.20	.14	.13	.48	.78	3.01
Isoleucine	.52	.40	.42	.50	.36	.34	1.89	1.52	3.82
Leucine	1.05	.81	.84	.98	.73	.69	3.50	2.77	6.38
Tyrosine	.44	.26	.27	.34	.24	.24	1.28	1.05	2.90
Phenylalanine	.74	.53	.60	.71	.53	.47	2.43	1.71	4.56
Lysine	.47	.45	.43	.44	.39	.43	3.14	2.29	5.73
Histidine	.37	.23	.25	.34	.22	.21	1.23	1.01	-
Arginine	.75	.65	.56	.75	.53	.65	3.08	2.19	4.47
Total	14.94	9.88	10.83	13.32	9.11	8.31	41.73	34.04	73.62

[1]Amino acid content used for fecal digestibilities in Chapter 1.

[2]Amino acid content used for ileal digestibilities in the remaining chapters.

Table XII.2 Estimates of endogenous amino acid excretion from the ileum of chickens, obtained using the intercepts at zero amino acid intake of the linear regressions of amino acid excreted per 100 grams feed intake.

Amino acid	Wheat g	Oats g	Barley g
Aspartic acid	.031 ± .011[1]	.037 ± .013	.044 ± .007
Threonine	.029 ± .003	.027 ± .008	.039 ± .005
Serine	.030 ± .007	.037 ± .012	.035 ± .006
Glutamic acid	.026 ± .018	.037 ± .017	.040 ± .010
Proline	.023 ± .009	.024 ± .008	.026 ± .005
Glycine	.019 ± .009	.031 ± .013	.025 ± .005
Alanine	.019 ± .006	.017 ± .010	.023 ± .009
Valine	.020 ± .007	.018 ± .009	.026 ± .005
Methionine	.003 ± .002	.012 ± .007	.006 ± .002
Isoleucine	.011 ± .004	.017 ± .009	.018 ± .003
Leucine	.018 ± .007	.014 ± .007	.028 ± .005
Tyrosine	.017 ± .004	.021 ± .007	.024 ± .003
Phenylalanine	.028 ± .005	.028 ± .011	.038 ± .005
Lysine	.012 ± .006	.014 ± .008	.017 ± .004
Histidine	.005 ± .003	.003 ± .005	.009 ± .002
Arginine	.012 ± .008	.004 ± .014	.022 ± .007
Total	.306 ± .093	.340 ± .123	.420 ± .071

[1]Excreted amino acid ± standard error.

Table XII.3 Estimated grams amino acid excreted per 100 grams intake of a N-free diet obtained using the intercepts of the linear regressions for wheat-soybean meal and wheat-canola meal.

Amino acid	Wheat - Soybean meal		Wheat - Canola meal	
	Ileal	Fecal	Ileal	Fecal
Aspartic acid	.080 ± .012[1]	.178 ± .019	.053 ± .017	.182 ± .018
Threonine	.059 ± .008	.118 ± .012	.040 ± .011	.121 ± .012
Serine	.050 ± .008	.125 ± .013	.035 ± .009	.127 ± .013
Glutamic acid	.082 ± .018	.220 ± .025	.040 ± .024	.222 ± .025
Proline	.052 ± .013	.123 ± .018	.040 ± .014	.121 ± .029
Glycine	.033 ± .008	-	.018 ± .011	-
Alanine	.031 ± .008	.086 ± .009	.026 ± .006	.090 ± .011
Valine	.035 ± .011	.098 ± .012	.021 ± .016	.099 ± .014
Methionine	.012 ± .004	.030 ± .004	.009 ± .003	.029 ± .006
Isoleucine	.029 ± .007	.075 ± .008	.020 ± .007	.078 ± .009
Leucine	.044 ± .011	.127 ± .012	.029 ± .011	.133 ± .012
Tyrosine	.033 ± .016	.105 ± .018	.014 ± .025	.104 ± .020
Phenylalanine	.059 ± .009	.113 ± .014	.048 ± .020	.115 ± .018
Lysine	.029 ± .010	.097 ± .011	.014 ± .009	.104 ± .010
Histidine	.008 ± .005	.018 ± .008	.003 ± .001	.015 ± .010
Arginine	.018 ± .014	.081 ± .010	.016 ± .009	.084 ± .009
Total	.678 ± .139	1.740 ± .184	.421 ± .160	1.762 ± .200

[1]Intercept ± standard error.

Table XII.4 Quadratic regressions of ileal grams digested amino acid versus amino acid intake per 100 grams feed-intake for diets containing wheat-soybean meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	-.079 ± .014[1]	.852 ± .042	.006 ± .023
Threonine	-.063 ± .009	.933 ± .063	-.084 ± .086
Serine	-.053 ± .009	.918 ± .044	-.030 ± .041
Glutamic acid	-.082 ± .021	.935 ± .023	-.001 ± .001
Proline	-.051 ± .015	.891 ± .062	.009 ± .047
Glycine	-.038 ± .009	.903 ± .055	-.077 ± .063
Alanine	-.033 ± .009	.893 ± .052	-.027 ± .057
Valine	-.034 ± .013	.818 ± .085	.023 ± .104
Methionine	-.010 ± .005	.798 ± .097	.256 ± .372
Isoleucine	-.029 ± .008	.886 ± .054	-.013 ± .066
Leucine	-.047 ± .012	.903 ± .043	-.012 ± .028
Tyrosine	-.031 ± .018	.759 ± .177	.087 ± .317
Phenylalanine	-.061 ± .011	.905 ± .052	-.015 ± .047
Lysine	-.032 ± .011	.920 ± .056	-.028 ± .051
Histidine	-.006 ± .006	.860 ± .063	.056 ± .115
Arginine	-.035 ± .017	.880 ± .070	.034 ± .054

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.5 Quadratic regressions of fecal grams digested amino acid versus amino acid intake per 100 grams feed intake for diets containing wheat-soybean meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	-.203 ± .016[1]	1.056 ± .047	-.084 ± .026*
Threonine	-.136 ± .008	1.242 ± .056	-.387 ± .076*
Serine	-.146 ± .008	1.175 ± .039	-.202 ± .036*
Glutamic acid	-.256 ± .019	1.041 ± .021	-.017 ± .004*
Proline	-.153 ± .008	1.198 ± .033	-.200 ± .025*
Glycine	-	-	-
Alanine	-.097 ± .009	1.058 ± .051	-.153 ± .056*
Valine	-.114 ± .010	1.142 ± .068	-.283 ± .083*
Methionine	-.035 ± .004	1.135 ± .081	-.912 ± .310*
Isoleucine	-.087 ± .005	1.102 ± .033	-.201 ± .041*
Leucine	-.145 ± .008	1.081 ± .029	-.088 ± .018*
Tyrosine	-.128 ± .015	1.505 ± .144	-.847 ± .258*
Phenylalanine	-.135 ± .008	1.165 ± .041	-.208 ± .038*
Lysine	-.111 ± .010	1.087 ± .050	-.131 ± .046*
Histidine	-.021 ± .009	.997 ± .089	-.120 ± .163
Arginine	-.092 ± .010	1.053 ± .041	-.073 ± .032*

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.6 Quadratic regressions of ileal grams digested amino acid versus amino acid intake per 100 grams feed intake for diets containing wheat-canola meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	-.070 ± .019[1]	.814 ± .047	-.041 ± .023
Threonine	-.058 ± .009	.894 ± .043	-.139 ± .037*
Serine	-.049 ± .009	.881 ± .035	-.838 ± .028*
Glutamic acid	-.076 ± .021	.949 ± .021	-.014 ± .004*
Proline	-.040 ± .017	.794 ± .045	-.001 ± .024
Glycine	-.035 ± .009	.885 ± .036	-.098 ± .027*
Alanine	-.029 ± .007	.860 ± .025	-.017 ± .019
Valine	-.029 ± .019	.800 ± .076	-.048 ± .059
Methionine	-.007 ± .003	.842 ± .037	-.057 ± .070
Isoleucine	-.025 ± .008	.846 ± .038	-.039 ± .036
Leucine	-.042 ± .010	.897 ± .027	-.035 ± .014*
Tyrosine	-.023 ± .030	.830 ± .212	-.171 ± .285
Phenylalanine	-.055 ± .011	.880 ± .045	-.048 ± .036
Lysine	-.029 ± .006	.911 ± .021	-.071 ± .014*
Histidine	-.007 ± .009	.953 ± .070	-.151 ± .097
Arginine	-.029 ± .008	.962 ± .028	-.057 ± .018*

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.7 Quadratic regressions of fecal grams digested amino acid versus amino acid intake per 100 grams feed intake for diets containing wheat-canola meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	-.206 ± .015[1]	.971 ± .041	-.064 ± .020*
Threonine	-.137 ± .009	1.043 ± .042	-.144 ± .037*
Serine	-.147 ± .010	1.070 ± .041	-.140 ± .041*
Glutamic acid	-.258 ± .019	1.012 ± .020	-.015 ± .004*
Proline	-.153 ± .028	1.048 ± .078	-.099 ± .042*
Glycine	-	-	-
Alanine	-.098 ± .012	.962 ± .048	-.049 ± .036
Valine	-.116 ± .013	1.016 ± .056	-.115 ± .044*
Methionine	-.034 ± .007	1.028 ± .068	-.210 ± .132
Isoleucine	-.088 ± .008	1.007 ± .040	-.100 ± .038*
Leucine	-.148 ± .010	1.369 ± .026	-.043 ± .013*
Tyrosine	-.131 ± .016	1.097 ± .113	-.564 ± .154*
Phenylalanine	-.135 ± .017	.954 ± .073	-.141 ± .059*
Lysine	-.112 ± .010	.994 ± .037	-.040 ± .025
Histidine	-.021 ± .012	1.017 ± .087	-.120 ± .122
Arginine	-.095 ± .007	1.028 ± .024	-.053 ± .016*

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.8 Estimated grams amino acid excreted per 100 grams intake of a N-free diet obtained using the intercepts of the linear regressions for soybean meal and canola meal.

Amino acid	Soybean meal		Canola meal	
	Ileal	Fecal	Ileal	Fecal
Aspartic acid	.014 ± .046[1]	.099 ± .030	.013 ± .038	.134 ± .036
Threonine	.029 ± .017	.071 ± .015	.019 ± .021	.076 ± .020
Serine	.022 ± .018	.071 ± .013	.009 ± .024	.078 ± .020
Glutamic acid	.011 ± .053	.124 ± .040	.002 ± .051	.151 ± .043
Proline	.006 ± .026	.085 ± .024	.004 ± .031	.088 ± .038
Glycine	.010 ± .017	-	.013 ± .030	-
Alanine	.011 ± .018	.055 ± .013	.001 ± .023	.064 ± .020
Valine	.003 ± .021	.053 ± .015	.019 ± .031	.059 ± .027
Methionine	.001 ± .001	.029 ± .006	.008 ± .010	.030 ± .009
Isoleucine	.008 ± .016	.051 ± .012	.032 ± .020	.053 ± .018
Leucine	.011 ± .026	.066 ± .021	.066 ± .033	.075 ± .025
Tyrosine	.013 ± .012	.047 ± .010	.001 ± .018	.048 ± .016
Phenylalanine	.022 ± .021	.067 ± .017	.011 ± .024	.078 ± .021
Lysine	.015 ± .013	.063 ± .015	.005 ± .023	.072 ± .020
Histidine	.007 ± .010	.012 ± .008	.002 ± .008	.021 ± .007
Arginine	.013 ± .021	.042 ± .016	.008 ± .015	.051 ± .012
Total	.185 ± .294	.932 ± .224	.022 ± .342	1.077 ± .316

[1]Intercept ± standard error.

Table XII.9 Quadratic regressions of ileal grams digested amino acid versus amino acid intake per 100 grams feed intake for diets containing soybean meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	-.083 ± .036[1]	.982 ± .051	-.054 ± .014*
Threonine	-.059 ± .006	1.032 ± .023	-.206 ± .019*
Serine	-.053 ± .010	.988 ± .030	-.111 ± .018*
Glutamic acid	-.089 ± .040	.995 ± .038	-.027 ± .007*
Proline	-.052 ± .010	1.041 ± .036	-.238 ± .026*
Glycine	-.038 ± .011	.998 ± .045	-.181 ± .036*
Alanine	-.039 ± .013	1.009 ± .041	-.107 ± .026*
Valine	-.031 ± .018	1.022 ± .079	-.211 ± .070*
Methionine	-.009 ± .010	.992 ± .144	-.684 ± .433
Isoleucine	-.033 ± .012	.996 ± .045	-.143 ± .034*
Leucine	-.052 ± .017	.989 ± .035	-.070 ± .014
Tyrosine	-.029 ± .010	.982 ± .059	-.205 ± .067*
Phenylalanine	-.052 ± .016	1.007 ± .048	-.104 ± .029*
Lysine	-.035 ± .010	.974 ± .023	-.042 ± .010
Histidine	-.011 ± .012	.919 ± .073	-.062 ± .087
Arginine	-.025 ± .025	.923 ± .059	-.027 ± .028

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.10 Quadratic regressions of fecal grams digested amino acid versus amino acid intake per 100 grams feed intake for diets containing soybean meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	-.144 ± .023[1]	.979 ± .033	-.036 ± .009*
Threonine	-.090 ± .014	1.011 ± .059	-.127 ± .049*
Serine	-.091 ± .010	.994 ± .031	-.074 ± .019*
Glutamic acid	-.187 ± .026	.998 ± .025	-.022 ± .005*
Proline	-.095 ± .028	.901 ± .103	-.050 ± .075*
Glycine	-	-	-
Alanine	-.070 ± .012	.968 ± .038	-.060 ± .024*
Valine	-.070 ± .015	.966 ± .065	-.125 ± .057
Methionine	-.030 ± .007	.874 ± .114	-.118 ± .341
Isoleucine	-.059 ± .014	.924 ± .055	-.049 ± .042
Leucine	-.097 ± .017	.996 ± .035	-.052 ± .014*
Tyrosine	-.054 ± .011	.983 ± .061	-.130 ± .069
Phenylalanine	-.086 ± .018	.973 ± .055	-.059 ± .033
Lysine	-.078 ± .015	.959 ± .035	-.032 ± .016
Histidine	-.024 ± .007	1.031 ± .041	-.161 ± .048*
Arginine	-.063 ± .015	.985 ± .031	-.045 ± .044

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.11 Quadratic regressions of ileal grams digested amino acid versus amino acid intake per 100 grams feed intake for diets containing canola meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	-.075 ± .023[1]	.894 ± .042	-.085 ± .016
Threonine	-.056 ± .008	.900 ± .028	-.160 ± .019
Serine	-.050 ± .012	.933 ± .039	-.166 ± .025
Glutamic acid	-.080 ± .033	.979 ± .030	-.027 ± .005
Proline	-.047 ± .025	.846 ± .055	-.083 ± .025
Glycine	-.036 ± .018	.951 ± .052	-.170 ± .031
Alanine	-.038 ± .012	.990 ± .034	-.117 ± .019
Valine	-.035 ± .015	1.010 ± .045	-.197 ± .027
Methionine	-.010 ± .005	1.053 ± .037	-.365 ± .053
Isoleucine	-.032 ± .008	.970 ± .029	-.186 ± .022
Leucine	-.042 ± .010	.897 ± .027	-.035 ± .014
Tyrosine	-.030 ± .010	.963 ± .055	-.325 ± .058
Phenylalanine	-.051 ± .012	1.027 ± .040	-.168 ± .026
Lysine	-.031 ± .015	.945 ± .036	-.084 ± .018
Histidine	-.014 ± .006	.950 ± .033	-.143 ± .036
Arginine	-.028 ± .013	.955 ± .034	-.052 ± .017

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.12 Quadratic regressions of fecal grams digested amino acid versus amino acid intake per 100 grams feed intake for diets containing canola meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	-.138 ± .044[1]	.816 ± .083	-.005 ± .031
Threonine	-.090 ± .023	.888 ± .077	-.060 ± .051
Serine	-.090 ± .023	.884 ± .074	-.049 ± .048
Glutamic acid	-.186 ± .047	.959 ± .043	-.011 ± .008
Proline	-.094 ± .046	.829 ± .103	-.010 ± .046
Glycine	-	-	-
Alanine	-.071 ± .024	.916 ± .068	-.024 ± .038
Valine	-.074 ± .031	.930 ± .095	-.057 ± .058
Methionine	-.032 ± .011	.943 ± .077	-.039 ± .112
Isoleucine	-.062 ± .022	.917 ± .080	-.047 ± .059
Leucine	-.093 ± .028	.949 ± .105	-.008 ± .111
Tyrosine	-.049 ± .020	.841 ± .105	-.008 ± .111
Phenylalanine	-.086 ± .025	.943 ± .080	-.014 ± .028
Lysine	-.078 ± .024	.864 ± .057	-.014 ± .028
Histidine	-.026 ± .008	.938 ± .034	-.020 ± .017
Arginine	-.059 ± .013	.938 ± .034	-.020 ± .017

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.13 Amino acid digestibility obtained by linear regression for ileal, fecal and total fecal collections of soybean meal.

Amino Acid	Sample Ileal Digestibility %	Sample Fecal Digestibility %	Total Fecal Digestibility %
Aspartic acid	78.3 ± 1.5[1]	79.7 ± 1.9	86.2 ± .9
Threonine	79.0 ± 3.1	76.6 ± 3.6	83.1 ± 2.3
Serine	81.6 ± 1.4	84.9 ± 1.6	89.1 ± 1.1
Glutamic acid	83.4 ± 1.2	84.8 ± 1.6	87.3 ± 1.9
Proline	82.7 ± 2.0	82.3 ±2.39	87.2 ± 1.7
Glycine	80.9 ± 3.3	-	-
Alanine	80.6 ± 2.0	81.2 ± 2.3	83.7 ± 1.4
Valine	82.6 ± 2.2	81.0 ± 3.2	86.2 ± 3.2
Methionine	83.8 ± 2.0	79.9 ± 4.4	86.0 ± 6.5
Isoleucine	82.0 ± 1.6	83.1 ± 2.2	87.4 ± 1.1
Leucine	80.9 ± 1.5	82.4 ± 1.9	85.8 ± .92
Tyrosine	79.0 ± 2.4	77.2 ± 3.7	81.4 ± 8.6
Phenylalanine	81.5 ± 1.7	82.5 ± 2.4	97.3 ± 2.4
Lysine	85.0 ± .9	84.3 ± 1.5	89.2 ± 8.4
Histidine	85.5 ± 1.1	85.7 ± 1.2	90.1 ± 1.4
Arginine	85.5 ± 1.1	81.9 ± 1.9	89.0 ± 1.2

[1]Regression coefficient ± standard error.

Table XII.14 Quadratic regressions of ileal grams excreted amino acid versus amino acid intake per 100 grams feed intake for diets containing soybean meal.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	.100 ± .029[1]	.048 ± .034	.039 ± .007*
Threonine	.080 ± .024	-.032 ± .094	.176 ± .066*
Serine	.064 ± .011	.051 ± .029	.066 ± .014*
Glutamic acid	.110 ± .046	.035 ± .034	.019 ± .005*
Proline	.070 ± .017	-.068 ± .050	.134 ± .026
Glycine	.056 ± .026	-.014 ± .084	.131 ± .049*
Alanine	.072 ± .020	-.030 ± .067	.133 ± .040*
Valine	.046 ± .016	.021 ± .062	.112 ± .042*
Methionine	.012 ± .005	.123 ± .062	.103 ± .135
Isoleucine	.039 ± .013	.053 ± .047	.084 ± .029*
Leucine	.097 ± .024	-.017 ± .046	.071 ± .016*
Tyrosine	.033 ± .005	.130 ± .037	.113 ± .046*
Phenylalanine	.059 ± .015	.039 ± .047	.084 ± .026*
Lysine	.040 ± .011	.049 ± .024	.041 ± .009*
Histidine	.027 ± .005	.011 ± .029	.129 ± .027*
Arginine	.039 ± .014	.040 ± .031	.043 ± .012*

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.15 Quadratic regressions of fecal grams excreted amino acid versus amino acid intake per 100 grams feed intake for diets containing soybean meal.

Amino acid	b_0	b_1X	b_2X^2
Aspartic acid	.144 ± .038[1]	.004 ± .046	.046 ± .010*
Threonine	.092 ± .025	-.075 ± .098	.228 ± .069*
Serine	.098 ± .015	.002 ± .037	.074 ± .017*
Glutamic acid	.197 ± .054	-.017 ± .040	.025 ± .006*
Proline	.102 ± .018	-.068 ± .050	.134 ± .026*
Glycine	-	-	-
Alanine	.072 ± .020	-.030 ± .066	.133 ± .040*
Valine	.106 ± .015	-.030 ± .058	.100 ± .039*
Methionine	.031 ± .009	-.178 ± .121	.854 ± .260
Isoleucine	.065 ± .017	-.052± .058	.143 ± .036
Leucine	.097 ± .024	-.017 ± .046	.071 ± .016*
Tyrosine	.049 ± .010	-.012 ± .072	.314 ± .089
Phenylalanine	.086 ± .019	-.071 ± .060	.142 ± .034
Lysine	.080 ± .020	.032 ± .042	.051 ± .017
Histidine	.027 ± .006	.011 ± .029	.129 ± .027*
Arginine	.071 ± .024	-.003 ± .052	.075 ± .020

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.16 Quadratic regressions between the level of amino acid in the feces and amino acid intake per 100 grams feed intake for diets containing soybean meal using total collections.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	.135 ± .017[1]	.035 ± .021	.024 ± .005*
Threonine	.095 ± .023	.179 ± .087	.007 ± .062
Serine	.105 ± .015	.105 ± .037	.002 ± .017
Glutamic acid	.107 ± .076	-.043 ± .062	.024 ± .009*
Proline	.109 ± .022	.079 ± .059	.027 ± .031
Glycine	-	-	-
Alanine	.079 ± .016	.118 ± .053	.028 ± .032
Valine	.083 ± .033	.245 ± .117	.070 ± .080
Methionine	.032 ± .020	.521 ± .210	.871 ± .461
Isoleucine	.069 ± .012	.060 ± .040	.043 ± .025
Leucine	.098 ± .010	.042 ± .020	.037 ± .007*
Tyrosine	.051 ± .043	.609 ± .246	.550 ± .302
Phenylalanine	.095 ± .021	.120 ± .063	.004 ± .035
Lysine	.075 ± .014	.074 ± .030	.014 ± .012
Histidine	.016 ± .009	.012 ± .050	.085 ± .047
Arginine	.052 ± .016	.001 ± .037	.044 ± .015*

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.17 Logarithmic regressions between the level of amino acid in ileal and fecal sample collections and total fecal collections and amino acid intake per 100 grams feed of diets containing soybean meal.

Amino acid	Sample Ileal		Sample Fecal		Total fecal	
	b_0	b_1x	b_0	b_1x	b_0	b_1x
Aspartic acid	-2.00	.474	-2.08	.490	-1.57	.261
Threonine	-2.83	1.305	-2.93	1.485	-2.29	.772
Serine	-.2.59	.870	-2.53	.790	-2.15	.462
Glutamic Acid	-1.86	.305	-1.87	.301	-1.36	.168
Proline	-2.78	.976	-2.72	.994	-2.20	.548
Glycine	-2.88	1.132	-	-	-	-
Alanine	-2.90	1.163	-2.97	1.214	-2.38	.743
Valine	-3.03	1.272	-3.09	1.379	-2.39	.701
Methionine	-3.83	3.122	-4.16	4.400	-3.28	1.729
Isoleucine	-2.97	1.189	-3.14	1.285	-2.55	.705
Leucine	-2.53	.733	-2.58	.744	-2.03	.433
Tyrosine	-3.12	1.983	-3.25	2.254	-2.62	1.060
Phenyalanine	-2.74	1.018	-2.94	1.131	-2.34	.611
Lysine	-2.86	.809	-2.59	.748	-2.01	.333
Histidine	-3.97	2.145	-3.76	1.972	-3.18	.440
Arginine	-2.94	.824	-2.80	.869	-2.36	.440

Table XII.18 Dietary intakes of the experimental diets used in Chapters 3, 5 and 6.

Diet No.	Dietary Treatment	Feeding Period hr	Intake grams/chick
Chapter 4			
1	90% sucrose	12	54
2	90% wheat	12	65
3	60% wheat	12	89
4	30% wheat	12	87
5	90% oats	12	69
6	60% oats	12	68
7	30% oats	12	64
8	90% barley	12	62
9	60% barley	12	86
10	30% barley	12	80
Chapter 6			
1	86% cornstarch	12	100
1	22% SBM[1]	12	130
1	44% SBM	12	105
1	66% SBM	12	116
1	28% CM[2]	12	130
1	57% CM	12	94
1	86% CM	12	69
Chapter 7			
1	87% cornstarch	12	72
2	22% SBM	12	98
3	44% SBM	12	112
4	66% SBM	12	105
5	88% SBM	12	96
6	87% cornstarch	12	107
7	10% egg albumen	12	158
8	20% egg albumen	12	159
9	30% egg albumen	12	173
10	40% egg albumen	12	141

[1]Soybean meal

[2]Canola meal

Table XII.19 Quadratic regressions between the level of amino acid in ileal digesta and amino acid intake per 100 grams feed intake for diets containing egg albumen.

Amino acid	b_0	b_1x	b_2x^2
Aspartic acid	.139 ± .078[1]	-.245 ± .113	.206 ± .033*
Threonine	.085 ± .033	-.153 ± .111	.401 ± .075*
Serine	.090 ± .046	-.287 ± .099	.320 ± .044*
Glutamic acid	.151 ± .093	-.281 ± .106	.164 ± .025*
Proline	.076 ± .031	-.291 ± .125	.625 ± .102
Glycine	.058 ± .023	-.241 ± .101	.607 ± .088*
Alanine	.067 ± .043	-.289 ± .109	.363 ± .057*
Valine	.070 ± .050	-.287 ± .120	.350 ± .058*
Methionine	.028 ± .030	-.294 ± .116	.545 ± .093
Isoleucine	.057 ± .039	-.312 ± .120	.482 ± .075*
Leucine	.101 ± .091	-.356 ± .168	.271 ± .063*
Tyrosine	.030 ± .045	-.131 ± .182	.454 ± .151*
Phenylalanine	.078 ± .043	-.312 ± .112	.391 ± .059*
Lysine	.066 ± .045	-.250 ± .092	.258 ± .039*
Arginine	.058 ± .042	-.257 ± .112	.333 ± .060*

*Quadratic regressions significantly different from linear regressions $P < .05$.

[1]Regression coefficient ± standard error.

Table XII.20 Logarithmic regressions between the level of amino acid in ileal digesta and amino acid intake per 100 grams feed intake for diets containing egg albumen.

Amino acid	b_0	b_1x
Aspartic acid	-2.55	.885
Threonine	-2.77	1.629
Serine	-3.06	1.338
Glutamic Acid	-2.60	.742
Proline	-2.03	2.030
Glycine	-3.31	2.322
Alanine	-3.46	1.657
Valine	-3.41	1.604
Methionine	-4.87	3.373
Isoleucine	-3.64	2.071
Leucine	-3.25	1.229
Tyrosine	-3.79	2.574
Phenyalanine	-3.25	1.565
Lysine	-3.52	1.412
Arginine	-3.65	1.727